



## **Anticancer Effect of *Moringa oleifera* Leaf Extract on A2780 Ovarian Cancer Cell Line**

**Tolosa Daniel Angasa<sup>1</sup>, Yinuo Hong<sup>1</sup>, Ziqiang Ding<sup>1</sup> and Xianfeng Ding<sup>1\*</sup>**

<sup>1</sup>College of Life Sciences, Zhejiang Sci-Tech University, Hangzhou 310018, Zhejiang, China.

### **Authors' contributions**

This work was carried out in collaboration among all authors. Author TDA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors YH and XD managed the analyses of the study. Author ZD managed the literature searches. All authors read and approved the final manuscript.

### **Article Information**

DOI:10.9734/AJRIMPS/2020/v9i130140

#### Editor(s):

(1) Dr. Somdet Srichairatanakool, Chiang Mai University, Thailand.

#### Reviewers:

(1) Ghulam Hussain, University of Faisalabad, Pakistan.

(2) Azando Erick Virgile, University of Parakou, Republic of Benin.

(3) Amooru Gangaiah Damu, Yogi Vemana University, India.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/56210>

**Received 10 February 2020**

**Accepted 17 April 2020**

**Published 21 April 2020**

**Original Research Article**

### **ABSTRACT**

In this study we investigated anticancer effect of *Moringa oleifera* leaf extract on A2780 ovarian cancer cell line. The leave of *Moringa oleifera* were grinded into powder and the phytochemicals were extracted using 80% ethanol. For fractionation and partitioning process four kinds of solvents were used in order: Petroleum ether, Dichloromethane, Ethyl acetate and Water extract until the extract is colorless or lightened. For cell proliferation assay all samples were dissolved in DimethylSulfoxide. Anticancer effect of *Moringa oleifera* were tested on A2780 ovarian cell line with four different extracts using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide. The final result showed that on Petroleum Ether, Dichloromethane and Ethyl Acetate extracts as the concentration increases from 1 µg/ml, 2 µg/ml, 3 µg/ml, 4 µg/ml inhibited A2780, despite that in water extracts when the concentration decreases the inhibitory effect increases, however, Dichloromethane Extract has higher anticancer effect on A2780 ovarian cancer cell line with insignificant cytotoxicity to the normal cell.

\*Corresponding author: E-mail: [bdd114@163.com](mailto:bdd114@163.com);

**Keywords:** A2780 ovarian cancer cell line; *Moringa oleifera* leaf; MTT assay.

## 1. INTRODUCTION

*Moringa oleifera* is the most widely cultivated species in the genus *Moringa*, the only genus in the plant family Moringaceae [1]. *Moringa oleifera* is one of the 14 species of family Moringaceae, native to Africa, Southeast Asia, India, Arabia, South America, and the Pacific and Caribbean Islands [2]. Because *Moringa oleifera* has been naturalized in many tropic and subtropic regions worldwide, the plant is referred to by a number of names such as miracle tree, drumstick tree, horseradish tree, ben oil tree, and "Mother's Best Friend" [3]. Almost all parts of the plant are used culturally for its nutritional value, purported medicinal properties and for taste and flavor as a vegetable and seed. The leaves of *Moringa oleifera* can be eaten fresh, cooked, or stored as a dried powder for many months reportedly without any major loss of its nutritional value [4]. Ovarian cancer is the seventh-most common cancer, and eighth –most common cause of death from cancer in women in the world. World Cancer Report [5]. Ovarian cancer survival rates are much lower than other cancers that affect women Zayas-Viera et al. [6].

In this Study we investigated anticancer effect of *Moringa oleifera* leaf extract on A2780 ovarian cancer cell line. *Moringa* contains 46 bioactive compounds which help cells to neutralize free radicals, the leaves are highly nutritious, rich in vitamins A, C and E act as a good source of natural antioxidant [7] which included the cure of inflammation, cardiovascular, gastrointestinal, hematological and hepatorenal disorders. Singh, [8]. The main objective of this study was to observe the efficacy of *Moringa oleifera* leaf extract on A2780 ovarian cancer cell line. The leaf of *Moringa oleifera* were grinded to powder form and extracted using 80% methanol. For fractionation and partitioning process four kinds of solvents were used in order: Petroleum ether, Dichloromethane, Ethyl acetate and Water extract until the extract is colorless or lightened. For cell proliferation assay all samples were dissolved in Dimethylsulfoxide (DMSO). Anticancer effect of *Moringa oleifera* leaf extract were tested on A2780 ovarian cell line with four different extracts using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide. The Dichloromethane fraction of *Moringa oleifera* leaf extract was nontoxic to normal cell and have anticancer effect on A2780 ovarian cancer cell line [6].

## 2. MATERIALS AND METHODS

### 2.1 Collection of *Moringa oleifera* Leaves

The leave of *Moringa oleifera* were collected from the city of Chaozhou, Guangdong, Peoples republic of China. Geographically the latitude of the city is 23° 40' 12" N and Longitude is 116° 37' 48" E on degree minutes second (DMS) unit. This plant species is cultivated and occur in abundance, it is safe to use for research purpose. The leaf was authenticated by biologist in College of Life Science and Medicine, Zhejiang Sci-Tech University.

### 2.2 Cell Culture

The ovarian cancer A2780 cell lines were originated from the Central Laboratory of Obstetrics and Gynecology Hospital of Zhejiang University Medical College and their cell were purchased from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). (Trypsin (0.25% Trypsin with 0.002% EDTA) and DMEM High Sugar Medium were purchased from Hangzhou Genom Biomedical technology co.LTD. Fetal Bovine Serum (FBS) were purchased from Hangzhou Nuoyang Biotechnology co.LTD. A2780 cells were cultured and maintained in 1500 RMP medium supplemented with 10% Fetal Bovine Serum (FBS) in Dulbecco's modified Eagle's Medium (DMEM) High Sugar Medium. The cell was cultured in 37°C, 5% CO<sub>2</sub> by complete medium for, more than 24 h. Cells were usually sub-cultured after 3-4 days when it had reached about 80% confluence.

### 2.3 Preparation of *Moringa oleifera* Leaf Extraction and Phytochemical Fractionation

The leaves of *Moringa oleifera* were dried under 60°C of constant weight by air dry oven, crushing passed through a 60-mesh sieve and stored for use in dryer. 80% ethanol, solid: liquid = 1:20, 30 g of *Moringa oleifera* is extracted by 600 ml ethanol Table 1. Extraction were performed in reflux instrument for 1.5 h. The extraction process was repeated twice. The extract was filtered through filter paper and concentrated by a rotary evaporator to about 1/10 volume. For phytochemical fractionation four kinds of solvents were used in order: Petroleum ether (upper

**Table 1. Extraction of 80% ethanol leaf of *Moringa oleifera***

Extract	Initial weight of <i>Moringa oleifera</i> leaf(g)	Extraction Yield (ml)
80% Ethanol Leaf of <i>Moringa oleifera</i>	30 g	100 ml

layer), Dichloromethane (lower layer), ethyl acetate (lower layer) and water extract until the extract is colorless or lightened.

The extract phase of 1, 2 and 3 were concentrated by rotary evaporator until the liquid become thick paste. First extract phase was dissolved in Tert-Butanol, and second and third extract phases were dissolved in water by ultrasonic. In the last the solution was added into the tray, kept in -80°C cryogenic refrigerator for 12 h, then overnight Freeze dried by freeze drying machine. The extract was stored at 4°C until further use. Extraction yield (100 ml) were our first extraction phase from 30 g of *Moringa oleifera* and 80% ethanol of 600 ml Table 1.

#### 2.4 Cell Proliferation Assay

In this assay, all samples were dissolved in Dimethyl Sulfoxide (DMSO, 0.1% highest final concentration). A2780 cells were harvested from an exponentially growing cell culture that has reached 80% confluence. The cell counting was made by hemocytometer and a concentration of 2000 cells/well ( $2 \times 10^4$  cells/ml) in 100  $\mu$ l was seeded to each well of 96 well-plate with exception of few rows as a control for 24 hours in a CO<sub>2</sub> saturated incubator. The media in the wells were removed and replaced with media containing sample of different concentrations (1  $\mu$ g/ml, 2  $\mu$ g/ml, 3  $\mu$ g/ml and 4  $\mu$ g/ml) dissolved in DMSO. We dissolved the material at 10 mg/ml as our mother liquor. The MTT test have three times of adding liquid: 100  $\mu$ l cell, 10  $\mu$ l MTT-AB and 100  $\mu$ l MTT-C. Our goal was to control the concentration of the material in the cellular environment at 100  $\mu$ g/ml. 10  $\mu$ g of the material were added to the cellular environment. 10  $\mu$ g material is 1  $\mu$ l material mother liquor.

MTT assay was used for cell viability assessment according to manufacturer's instruction, dissolved MTT powder were mixed with MTT solvent to prepare MTT reagent (5 mg/ml). 10  $\mu$ l of MTT reagent at final concentration of 0.5 mg/ml to each well and mixed gently. The mixture was incubated for 4 hours in a CO<sub>2</sub> incubator. 100  $\mu$ L of Formazan Solubilization Solution were added into each well. The plates were shaken for 10 minutes to dissolve the

formazan crystals. Absorbance values at 575 nm was taken using microplate reader. Percentage cell viability was calculated for all four different kinds of extracts concentration using the following formula.

$$\text{Cell Viability} = \frac{\text{Absorbance of Treated Cell Culture with Extracts}}{\text{Absorbance of untreated cell culture as a control}} \times 100$$

#### 2.5 Statistical Analysis

Statistical analysis was performed using SPSS software (SPSS, Inc., Chicago, IL, USA). For comparisons between two samples, an unpaired two-tailed t-test was performed. A p-value of < 0.05 was considered statistically significant. The results are reported as the mean  $\pm$  SD. Statistical significance was assigned at P < 0.05 (\*), P < 0.01 (\*\*) or P < 0.001 (\*\*\*). All experiments were performed at least three times.

### 3. RESULTS AND DISCUSSION

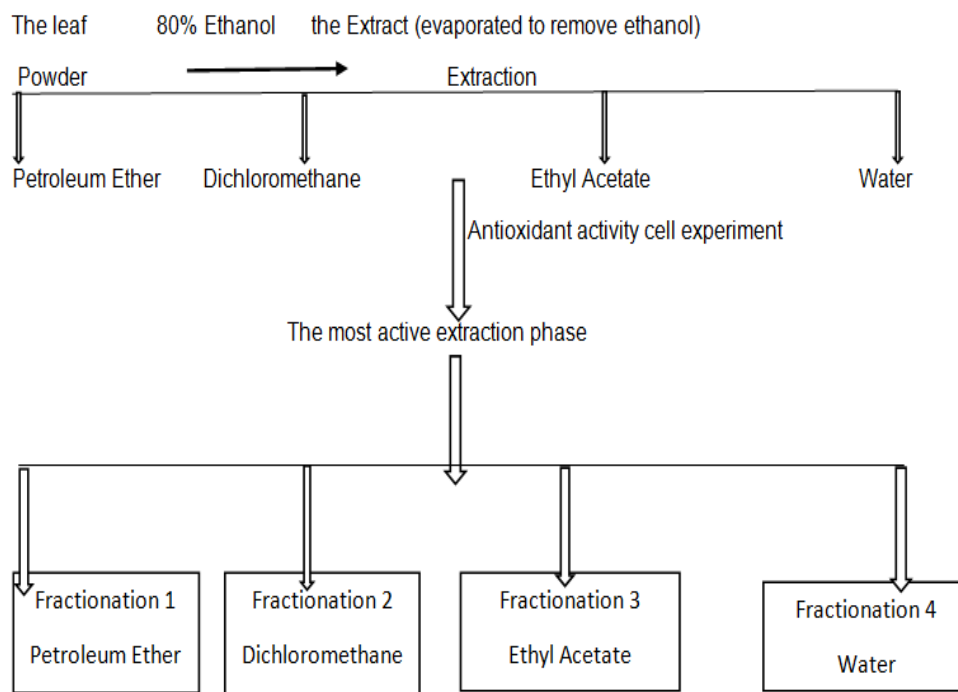
*Moringa* leaves are effective for the regulation of thyroid hormone status [9]. The methanol fraction of *Moringa oleifera* leaf extract showed antiulcerogenic and hepatoprotective effects in rats [10]. Extraction were performed in reflux instrument for 1.5 h. The extraction process was repeated twice Table 1. The extract was filtered through filter paper and concentrated by a rotary evaporator to about 1/10 volume. The ratio was solid: liquid = 1:20. Choice of ethanol was due to its higher solubility, strong extraction ability of plant phytochemicals and its tendency of yielding relevant compounds as it has been reported in previous studies on *Moringa oleifera* [11]. For phytochemical fractionation four kinds of solvents were used in order: Petroleum ether (upper layer), Dichloromethane (lower layer), ethyl acetate (lower layer) and water extract until the extract is colorless or lightened. In this phase we used all the solvents except water 800 ml, four times 200 ml each time until the extract is colorless the remaining water phase was left. The yield of all fractions was given in Table 2. This were the second phase of the experiment by chromatography the dichloromethane was the most active phase.

The extracts and fractions of *Moringa oleifera* Leaf were tested on A2780 Ovarian Cancer Cell line to determine the inhibitory and significant effects on cell proliferation (Fig. 2). Dichloromethane fraction had highest inhibitory effect on A2780 ovarian cancer cell line with insignificant cytotoxicity to the control of the experiment with the concentration varied from lower to higher the effect on the cell line (Fig. 2d). However, Petroleum Ether and Ethyl acetate fractions also had shown anticancer effect on A2780 while the concentration increases from 1 µg/ml, 2 µg/ml, 3 µg/ml and 4 µg/ml (Fig. 2 a; c). In addition to these three different fractions water fraction also shown some effect when the concentration decreases from 4 µg/ml to 1 µg/ml.

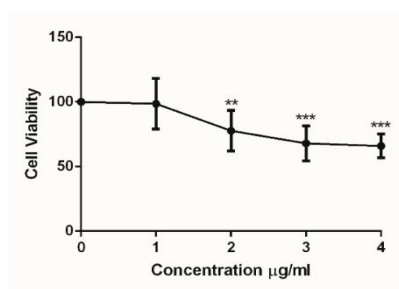
Inhibition of A2780 ovarian cancer cell line growth by Dichloromethane, Petroleum Ether and Ethyl Acetate fractions may be due to the antioxidant activities of phytochemical compounds all the samples contain. Our result only reveals the inhibition of cell proliferation. We believe additional mechanisms are needed to extend the study of this research. The main observations are consistent with several past works that have linked the anticancer activities of phenolic compounds to their antiproliferative effects on various cancer cells Diogo [12,13,14]. The Control and DMSO effect were determined (Fig. 2e). DMSO have no any cytotoxic effect on the cell line.

**Table 2. Fractionation of 80% ethanol extract of *Moringa oleifera* leaf**

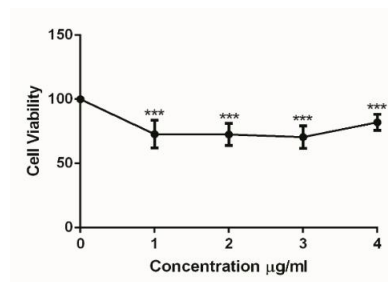
Fraction	Yield(g)	% Yield
Petroleum Ether	1.74	5.8
Dichloromethane	0.56	1.8
Ethyl Acetate	0.43	1.4
Water	7.1	23.6



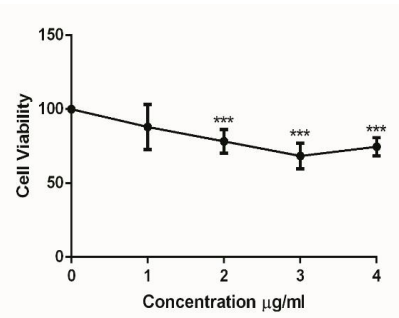
**Fig. 1. Schematic diagram on extraction and fractionation of *Moringa oleifera* leaf**



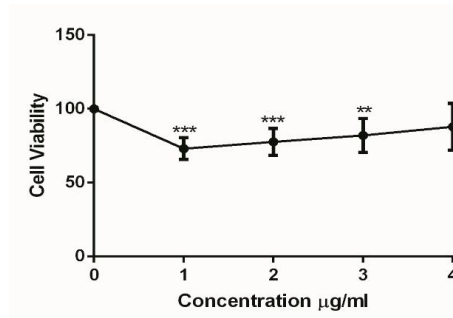
**A. Petroleum ether fraction effect on A2780**



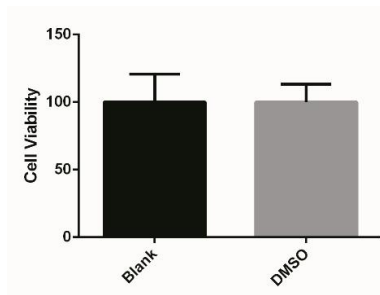
**B. Dichloromethane fraction effect on A2780**



**C. Ethyl acetate fraction effect on A2780**



**D. Water fraction effect on A2780**



**E. The untreated cell (control) and DMSO**

**Fig. 2. A; Petroleum ether B; Dichloromethane C; Ethyl Acetate D; Water fractions anticancer effect on A2780 Ovarian Cancer Cell Line E; Untreated cell as a control and DMSO effect on a normal A2780 cell line**

#### 4. CONCLUSION

In this study, different phytochemicals were extracted from *Moringa oleifera* Leaf, fractionated ethanolic extract on A2780 were determined, the final result showed that Dichloromethane fraction has higher anticancer effect on A2780 Ovarian cancer cell line with insignificant cytotoxicity to the normal cell (untreated cell) with the concentration varied from lower to higher the effect on the cell line. Anticancer effect of *Moringa oleifera* leaves on A2780 ovarian cancer cell line possibly will be due to the presence of antioxidants and different phytochemicals in the

leaves. However, in addition to this scientific study supplementary molecular investigation of action mechanisms sideways in cancer and *Moringa oleifera* are necessary.

#### DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of

knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

## ACKNOWLEDGEMENT

This research was supported by Zhejiang Sci-Tech University grants from the Zhejiang Natural Science Foundation of China (LY18C050006).

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. "Moringa oleifera". Germplasmre sources information network (GRIN). Agricultural research service (ARS), United States Department of Agriculture (USDA); 2017. [Retrieved 11 December 2017]
2. Iqbal S, Bhangar MI. Effect of season and production location on antioxidant activity of *Moringa oleifera* leaves grown in Pakistan. J. of Food Comp. and Anal. 2006;19:544-551.
3. Muluvi GM, Sprent JI, Soranzo N, Provan J, Odee D, Folkard G, McNicol JW, Powell W. Amplified fragment length polymorphism (AFLP) analysis of genetic variation in *M. oleifera* Lam. J. of Mol. Ecol. 1999;8:463-470.
4. Arabshahi-DS, Devi DV, Urooj A. Evaluation of antioxidant activity of some plant extracts and their heat, pH and storage stability. Food Chemistry. 2007; 100:1100-1105.
5. World Cancer Report. World Health Organization. 2014. Chapter 5.12. ISBN 978-9283204299. Archived from the original on 2016-09-19; 2014.
6. Zayas-Viera, María Del Mar, Vivas-Mejia, Pablo, Reyes, Jeyska. Anticancer effect of *Moringa oleifera* Leaf Extract in Human Cancer Cell Lines. Journal of Health Disparities Research and Practice. 2016; 9(5). [Article 102]
7. Siddhuraju P, Becker K. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agro-climatic origins of drumstick tree *Moringa oleifera* Lam. J Agric Food Chem. 2003;15:2144–2155.
8. Singh KK, Kumar K. Ethnotherapeutics of some medicinal plants used as antipyretic agent among the tribals of India. J Econ Taxon Bot. 1999;23:135–141.
9. Tahiliani P, Kar A. Role of *Moringa oleifera* leaf extract in the regulation of thyroid hormone status in adult male and female rats. Pharmacol Res. 2000;41:319–323.
10. Pal SK, Mukherjee PK, Saha BP. Studies on the antiulcer activity of *Moringa oleifera* leaf extract on gastric ulcer models in rats. Phytother Res. 1995;9:463–465.
11. Guevara AP, Vargas C, Sakurai H, Fujiwara Y, Hashimoto K, Maoka T, Kozuka M, Ito, Y, Tokuda H, Nishino H. An antitumor promoter from *Moringa oleifera* Lam. Mutation Research/ Genetic Toxicology and Environmental Mutagenesis. 1999;440(2):181-188.
12. Diogo CV, Félix L, Vilela S, Burgeiro A, Barbosa IA, Carvalho MJ, Oliveira PJ, Peixoto FP. Mitochondrial toxicity of the phytochemicals daphnetoxin and daphnoretin—Relevance for possible anticancer application. Toxicology *In vitro*. 2009;23(5):772-779.
13. Goel A, Kunnumakkara AB, Aggarwal BB. Curcumin as —CurecuminII: From kitchen to clinic. Biochemical Pharmacology. 2008;75(4):787-809.
14. Saeidnia S, Abdollahi M. Antioxidants: Friends or foe in prevention or treatment of cancer: Toxicology and Applied Pharmacology. 2013;271(1):49-63.

© 2020 Angasa et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

The peer review history for this paper can be accessed here:  
<http://www.sdiarticle4.com/review-history/56210>