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Study Anticancer effects of *Moringa oleifera* Leaf Water Extract on MCF-7 Breast-cancer Cells and Using its Leaves Powder as Food Additive

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Abstract

Breast cancer is the most common cancer among women, representing 23% of the total new cases and the second leading cause of cancer death in women. Natural medicines have been proven to be a good source of narrative agents with a pharmaceutical potential. *Moringa oleifera* consists of diverse plant chemicals that exhibit anti-cancer action through cytotoxic effects on various cancer cells. The objectives of the present study are to explore the effects of natural compounds of *Moringa oleifera* leaf water extract (MoWE) on the proliferation of MCF-7 cells. The second objective is to investigate the possibility of using *Moringa oleifera* (whole leaf) as natural food additives in some food products (gruel and biscuits). Results showed that MoWE possesses antioxidant scavenging DPPH activity (74.53%). Total polyphenolics, flavonoids and tannins were 44.77 mg GAE/ml, 5.86 and 22.16 mg QE /ml, respectively. The MoWE also exerted cytotoxic effect on MCF-7 cells with IC50 = 600µg/ml. MoWE induced down regulation of Bcl-2 and up-regulation of Bax proteins expression levels. The elevation of fourteen folds in Bax/Bcl-2 ratio was recorded in MoWE-treated groups. These results suggest that Moringa leaf water extract may have beneficial effects for the reduction of breast cancer growth, and new therapeutic strategy for the treatment of human cancers. The nutritive values of both gruel and biscuit were improved. The proximate chemical composition of gruel in the sample-3 (7.5%) was contained 21.86% moisture, 8.85 % protein, 5.28 % fat, 68.48% carbohydrate, 2.28% ash. While the proximate chemical composition of gruel in the sample-3 (7.5%) was contained 7.6% moisture, 10.21 % protein, 6.21 % fat, 73.56% carbohydrate, 1.8% ash. So *Moringa oleifera* leaf powder can be used as food additives to elevate the nutritive value of children foods.

Keywords: MCF-7, *Moringa oleifera*, Antioxidant, Anticancer, Apoptosis.

Introduction

Cancer is a major burden of disease which severely effects the human population worldwide. According to report of the **World Health Organization report (WHO), 2012,** cancer was the leading cause of death in economically developed countries and the second leading cause of death in developing countries. The global burden of cancer continues to increase because of the aging and growth of the world population, along with an increasing adoption of cancer-causing behaviors (**Jemal *et al*., 2011**). It is estimated that 14.1 million new cases and 8.2 million cancer deaths occurred in 2012 worldwide with lung and breast cancers being the most frequently diagnosed cancers and the leading causes of death among both men and women (**Torre *et al*., 2015**).

Breast cancer is defined as an uncontrolled spread of malignant tissue within the breast that must be surgically removed as it can eventually lead to death if growth is widespread. Breast tumors can be classified by their place of origin into a channel or lobular or inflammatory building. Breast cancer can be detected through several early symptoms, including regular discharge from the nipple and the nipples becoming inverted. Scientific research is now concerned with naturally derived compounds because they have less toxic side effects than current treatments such as chemotherapy (**American Cancer Society, 2017**).

Various types of plants have been used for several centuries worldwide not only as a dietary supplements, but also as traditional treatments for many diseases (**Wood, 1997; Iqbal & Bhanger, 2006 and Khalafalla *et al*., 2010**). Indeed, scientific and research interest is drawing its attention towards naturally-derived plant compounds as sources of bioactive compounds, including potential antitumor (**Elkhateeb *et al*., 2018 and El-Garawani *et al*., 2019**), antioxidant (**El-Nabi *et al*., 2018**), and antigenotoxic (**Sakr *et al*., 2016**) as well as having less toxic side effects compared to current treatments such as chemotherapy.

Among these plants, the widely cultivated *Moringa oleifera* (Moringa or drumstick tree) a rapidly growing perennial tree was used by the ancient Romans, Greeks, and the Egyptians. It was naturalized from the tropics to the sub- Himalayan regions (e.g. India, Pakistan, Bangladesh, Afghanistan, Oceania, Latin America, Africa and tropical Asia (**Oliveira *et al*., 1999; Fuglie, 1999; Fahey, 2005 and Mukunzi *et***

***al*., 2011**). Additionally, besides being edible, all the parts of the Moringa tree (e.g. pods, seeds, and leaves) have long been employed for the treatment of many diseases and therefore, it was called a ‘‘miracle vegetable’’ (**Faizi, *et al*., 1995; Fuglie, 1999 and Anwar *et al*., 2007**). Moringa is considered one of the most nutritious plants on the planet. It contains fats, high amounts of protein and carbohydrates, folate, calcium, phosphorous, beta-carotene, vitamin C, vitamin E, iron, potassium, and magnesium (**Mahmood *et al*., 2010 and Mukunzi *et al*., 2011**). For these reasons, some parts of this plant have drawn much attention and have been studied for its various biological activities, including antiatherosclerotic (**Chumark *et al*., 2008**), immune-boosting (**Miyoshi *et al*., 2004**), anticardiovascular diseases (**Faizi *et al*., 1994**), antiviral (**Khalafalla *et al*., 2010 and Waiyaput *et al*., 2012**), antioxidant (**Iqbal & Bhanger, 2006; Sultana *et al*., 2009 and Kumar *et al*., 2012**), antimicrobial, anti-inflammatory (**Kumar *et al*., 2012**), properties and tumor suppressive effects in skin papillomagenesis, hepatocarcinoma cancer, colon cancer, and myeloma (**Khalafalla *et al*., 2010; Brunelli *et al*., 2010 and Budda *et al*., 2011**).

Additionally, most studies have been conducted using solvent extracts of MOL and not their soluble extracts. Solvent extraction is the most frequently used technique for the isolation of bioactive compounds from plants. Therefore, the recovery of bioactive compounds from *Moringa oleifera* has been typically accomplished using various solvents, such as methanol and ethanol, as well as hot water and buffers (**Sultana *et al*., 2009; Khalafalla *et al*., 2010; Budda *et al*., 2011 and Kumar *et al*., 2012**). Nevertheless, the majority of the previous studies focus on solvent extracts because the efficacy of solvent extraction is higher than simple water extraction. The present study focuses on examining the possible anticancer effect of the water extract of *Moringa oleifera* leaves on MCF-7 breast cancer cells and investigate the possibility of using *Moringa oleifera* (whole leaf) as natural food additives in some food products (gruel and biscuits).

Materials and methods

Plants

Fresh leaves of moringa (*Moringa oleifera*, family: Moringacia) were collected freshly in June, 2017 from Egyptian Scientific Association of Moringa, National Research Center, Giza, Egypt.

Preparation of Moringa oleifera leaves

Leaves of *Moringa oleifera* were collected and washed under running tap water to eliminate dust and other foreign particles, then dried in shade at room temprature for one week then crushed to fine powder using domestic blender. Powdered was stored in polyethylene plastic bags until analysis (**Mishra *et al*., 2012**).

Preparation of Moringa oleifera aqueous extract

Leaves were separated from the bark. Two hundred grams of green Moringa leaves were immersed in 500 ml of hot water at 55 ° C for 24h., and then crushed with a domostic blender. The mixture was filtered by using Whatman filter paper (No.1) twice times to dispose of the fiber. The suspension was left overnight in the refrigerator. The supernatant was then filtered using Whatman filter paper (No.1). The supernatant was dried in oven at 55°C for two hrs. Finally, the dried *Moringa oleifera* water extract (MoWE) was stored at -20°C until use (**El-Garawani, 2015**).

Preparation of Moringa oleifera foods:

Moringa oleifera leaf powder was added to the flour. Make five blends according to the percentage. According to **Arise *et al*., 2014**, mixtures represent 1-5 wheat and dried *Moringa oleifera* leaves (DMOL) in proportions: 100: 0, 97.5: 2.5, 95: 5, 92.5:7.5and 90: 10 gm respectively. The biscuits and porridge were commercially prepared from these combinations and then analyzed chemically and sensitively.

Sensory evaluation of Control and DMOL gruel and biscuit samples

Sensory evaluation of biscuits was carried out by 14 panels (nursing mother). Four samples of biscuits in four replicates were evaluated by each panel following a score card consisting of various quality parameters like surface color, surface cracking pattern, crumb color, texture, mouth feel and flavor. The scores assigned in the score card for these parameters. The panel members were requested in measuring the terms identifying sensory characteristics and in use of the score. Judgments were made through rating products on a five-point Hedonic Scale with corresponding descriptive terms ranging from 9 ‘like extremely’ to 1 ‘dislike extremely (**Larmond, 1977**).

Proximate chemical analysis:

Moisture, crude fat, crude protein, total carbohydrates and ash of *Moringa oleifera* leaves and different blends were estimated according to (**A.O.A.C. 2010**).

Minerals contents:

Calcium, potassium, iron and other minerals were determined by emission measurements obtained by direct nebulization in an inductively coupled plasma optical emission spectrometer (ICP- OES), Baird model 2070 ICP(USA), with 100 cm optical length Czerny Turner monochromator (Thermo– Elmental, Model 300VA, UK, 1969).

Determination of total flavonoids

Total flavonoids content was determined and the results were expressed as mg/ml quercetin equivalents (QE) per 100 g sample (**Chen and Li, 2007**).

Determination of total polyphenolic compounds

Total phenolic content was determined depending on the Folin-Ciocalteau method (**Slinkard and Singleton, 1977**). Results were expressed as mg/ml of gallic acid equivalents (GAE) per 100 g sample.

Determination of antioxidant activities using DPPH radical-scavenging assay

Determination of the DPPH radical-scavenging activity depends on the method of **Brand-Williams *et al*., (1995**). Results were expressed as µg/ml of gallic acid equivalents (GAE) per 100 g sample. All phytochemical determinations were performed in triplicates.

MCF-7 cell line culturing

MCF-7 cells viability was evaluated according to trypan blue (0.4 %) stain method. This method is based on the principle that live (viable) cells do not take up certain dyes, whereas dead (non-viable) cells do. Cells count was calculated by (Cells / ml = 10^4 × (Average count per square) × (Dilution factor)). Cells were maintained in complete growth medium Roswell Park Memorial Institute medium (RBMI). [RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin (100 U/ml)/streptomycin (100 µg/ml). 5×10^5 cells were grown in each T25 culture flask containing 7ml of complete growth medium to reach 70% confluency in a humidified atmosphere of 5 % CO_2 at 37°C. All culture reagents were obtained from (Lonza) supplier, Egypt.

Morphological Examination by phase contrast inverted microscopy

Treated cells and controls were examined for morphological changes using (Olympus BX41, Japan) at 400× magnification and then representative photos were digitally captured.

Determination of cytotoxicity in MCF-7 cells

Cytotoxic effect of the extract was assessed using tetrazolium based colorimetric (MTT) assay. For the MTT assay, MCF-7 breast cancer cells (100 µl) were seeded at 1×10^5 cells/ml concentration into 96-well plates and incubated overnight in a humidified chamber at 37ºC in the presence of 5% CO_2 for 24 hr. The MCF-7 cells were then treated with concentrations of 0 - 1000 µg/ml serial dilutions of the extract in triplicates and incubated for 24 hr. After incubation, the cells were treated with 20 µl of 2.5 mg/ml MTT solution and incubated for 4 hr as indicated above. The samples were subsequently treated with 150 µl of acidified isopropanol and the plates had been incubated in the dark for 4 hours at room temperature (26ºC) to dissolve formazan crystals. The optical density was read using a spectrophotometer (Tecan Infinite M200 Pro plate reader, Austria) at 570 nm. The percent viability was determined and the Median inhibitory concentrations (IC_{50}) were determined. The dose of 150µg/ml was selected to investigate the mode of anticancer effect of the MoWE **(Hansen *et al.*, 1989).**

Giemsa staining

After various incubations, treated cells and controls were washed with PBS and fixed in a solution of 3 parts methanol: 1 part glacial acetic acid for 15 minutes, then washed with PBS for 1 minute and stained with Giemsa solution for 15 minutes followed by PBS washing. Five hundred cells were examined (400 ×) using a light inverted microscope (Olympus IMT-2, Japan) and digitally photographed (**Thippeswamy and Salimath, 2006).**

Bax and Bcl-2 protein expression

Immunocytochemical reaction was performed using an avidin biotin complex immunoperoxidase technique (**Hsu *et al.*, 1981**) with some modifications that smeared cells processed for immunocytochemical reaction instead of growing on coverslip on smeared cells of control and treated groups. Bcl-2 and Bax, as cytoplasmic markers for apoptosis, were detected using an anti-human Bcl-2 and Bax monoclonal antibodies (Glostrup). Bax/Bcl-2 ratio was determined. Cells were examined at 400× using light microscope (Olympus BX 41, Japan) and digitally photographed.

Flow cytometric analysis of cell cycle distribution

For cell cycle analysis, breast cancer cells were grown overnight and then treated with Moringa water extract. After 24 h of incubation, MCF-7 cells were trypsinized and fixed in 70% ice-cold ethanol at 4^oC

for 10 minutes. After incubation, cell pellet was washed and re-suspended in propidium iodide (PI) staining buffer and incubated at 37^{o}C for 15 minutes and then the percentages of cells in the different phases of cell cycle were evaluated by determining the PI stained DNA contents by FACS scan flow cytometer (Becton Dickinson, USA) **(Dobashi *et al*., 2003)**.

Statistical analysis

Data are presented as mean ± S.D. using SPSS. Student's *t*-test was used to assess any significant difference between each treated group compared to control group. One-way analysis of variance (ANOVA) was used to assess any significant difference between groups after incubation with treatments. The level of significance was set at $P < 0.05$ (**McClave and Dietrich, 1991**).

Results

Total phenolic compounds, tannins, flavonoid contents and antioxidant activity of MoWE

Moringa oleifera possessed 44.77, 5.86 and 22.16 (mg GAE/ml) for total phenolic compounds, tannins and flavonoid contents respectively. The DPPH scavenging assay revealed 74.53 % of MoWE antioxidant activity (**Table 1**).

Table 1: Flavonoid and total phenolic contents in *Moringa oleifera* water extracts

Parameter	MoWE
Total phenolic content (mg GAE/ml)	44.77
Tannins	5.86
Flavonid (mg QE/ml)	22.16
Antioxidant activity%	74.53

Cytotoxicity of plant extracts using MTT assay

The IC_{50} values obtained by the MTT assay were 600µg/ml for MoWE (**Table 2**) and the dose of 150µg/ml was specified to study the mechanistic anticancer properties of MoWE.

Table 2: The IC_{50} values of *Moringa oleifera* water extract (MoWE) after MTT assay on MCF-7 cells.

M.oWE µg/ml	Cytotoxicity
0	100
100	81.3
200	80.1
400	61.9
600	51
800	42
1000	39.7

Morphological changes in MCF-7 cells

The marked abnormal changes in cell morphology, such as irregular cell boundary, shrinkage and detaching, were observed in treated cells using phase contrast inverted light microscope (**Photo 1**). Moreover, Giemsa staining revealed the formation of cytoplasmic vaculation and membrane blebbing. It is the characteristic features of dead and apoptotic cells in MoWE-treated groups (**Photo 2**). The MCF-7 cells treated with MoWE recorded significant increase ($P \leq 0.05$) in damaged cells after 24 hr when compared with non-treated group (**Figure 1**).

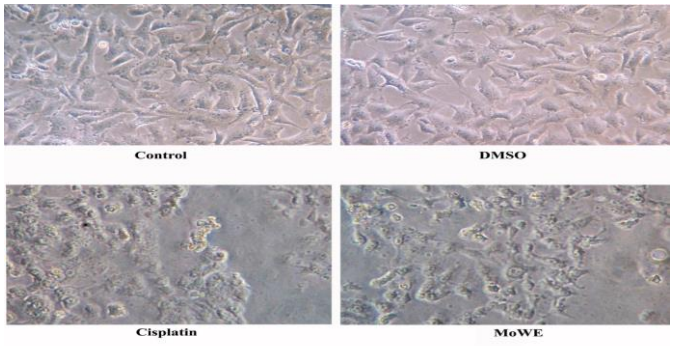

Photo-1: Photomicrographs of treated MCF-7 cells and controls for 24 hours (triplicate). The morphological changes were observed by inverted microscope (Phase contrast), Olympus IMT-2, Japan.

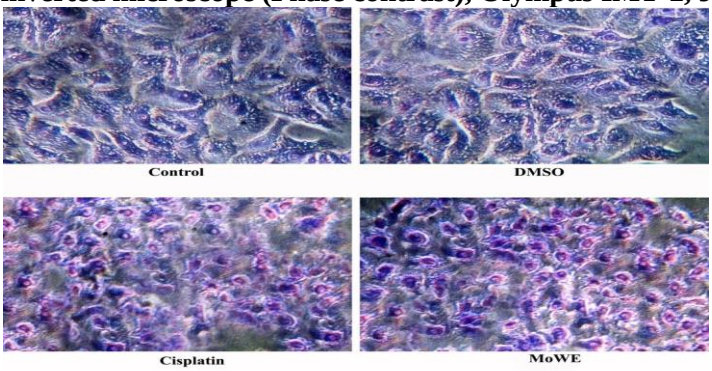

Photo2: Photomicrographs of treated MCF-7 cells and controls for 24 hours (triplicate). The morphological changes were observed (400×) after Giemsa staining by inverted microscope, Olympus IMT-2, Japan.

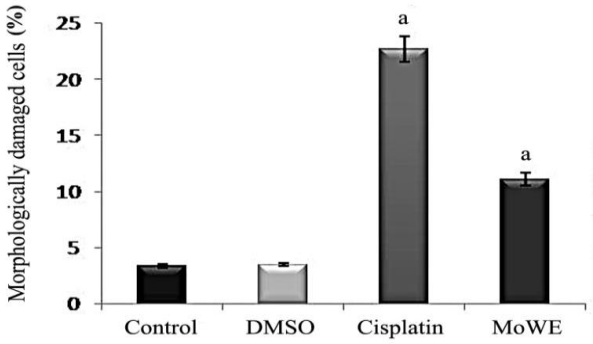

Figure-1: Percentage of morphologically damaged cell of treated MCF-7 cells and controls, for 24 hours, after Giemsa staining. A significant difference at ($P \leq 0.05$) with respect to untreated group. Data represent means of three different experiments; bars, standard deviation and a: significant ($P \leq 0.05$) with respect to the control (triplicate).

Cell cycle distribution

After incubation with desired concentrations of MoWE, cell cycle distribution was measured by flow cytometric analysis of DNA content after propidium iodide staining. The accumulation of cells with G_0/G_1 DNA content was apparent after 24 h of treatment in the cisplatin treated cells as a result of G_1 cell cycle arrest. Otherwise, doses of MoWE did not induce accumulation of MCF-7 population **(Figure 2).**

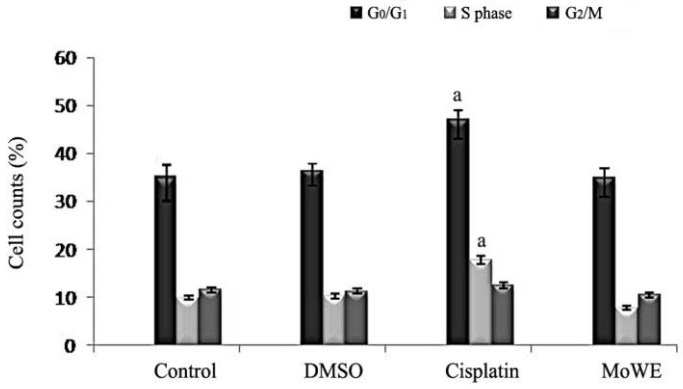

Figure-2: The effect of treatments on MCF-7 cell cycle distribution. The DNA content was evaluated with propidium iodide (PI) staining. Data represent means of three different experiments; bars, standard deviation and a: significant with respect to the control ($P \leq 0.05$).

Bcl-2 and Bax protein expression

The development of immunocytochemical reaction for Bcl-2 and Bax proteins was evaluated in treated and control cells **(Photo 3&4)** Results revealed that treated groups recorded significant down-regulation ($P \leq 0.05$) in the Bcl-2 protein expression and up-regulation of Bax protein in cells when compared with expression of untreated MCF-7 cells in a dose dependent manner **(Figure 3).** Bax/Bcl-2 ratio recorded an increased levels with treated groups leading to apoptosis association with the treatments **(Figure 4)**.

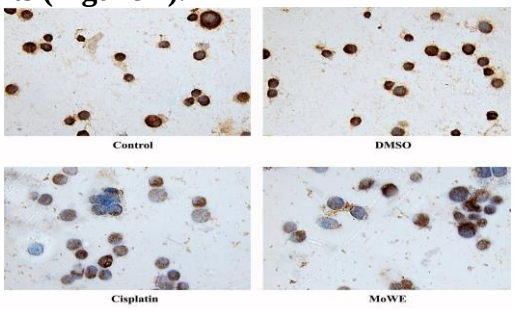

Photo-3: The effect of treatments on Bcl-2 protein expression as evaluated by immunocytochemical reactivity (+ve cells, brown staining), The results were obtained from three independent experiments (triplicate), (Olympus BX 41 microscope).

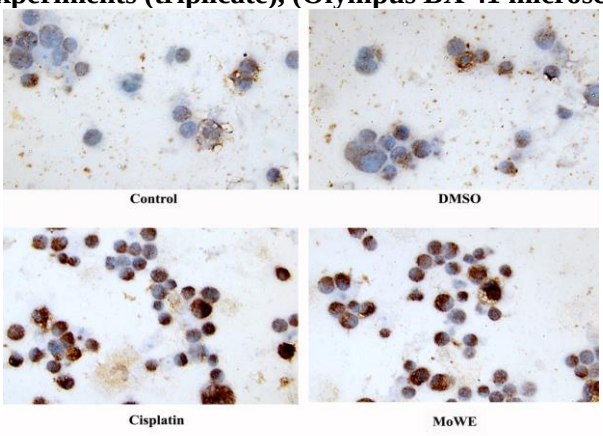

Photo-4: The effect of treatments on Bax protein expression as evaluated by immunocytochemical reactivity (+ve cells, brown staining). The results were obtained from three independent experiments (triplicate), (Olympus BX 41 microscope).

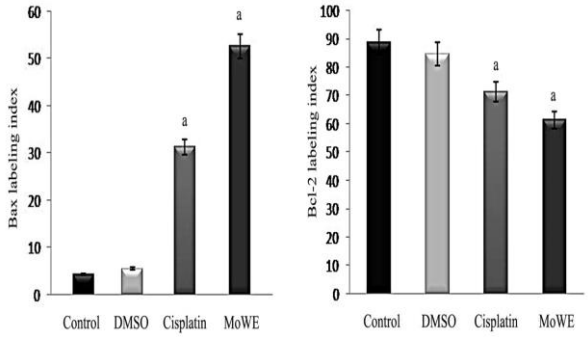

Figure-**3: Bcl-2 and Bax proteins expression as evaluated by immunocytochemical reactivity of treated MCF-7 cells and controls, for 24 hours. A significant difference at (*P*< 0.05) with respect to untreated group. Data represent means of three different experiments; bars, standard deviation and a: significant (*P* ≤ 0.05) with respect to the control (triplicate).**

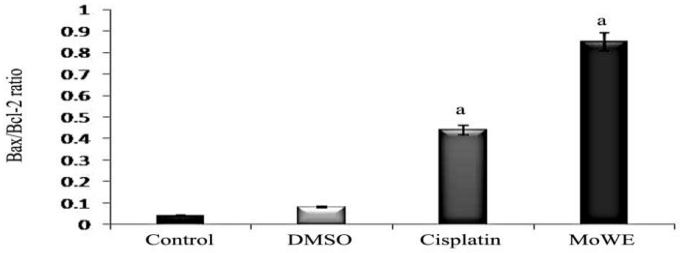

Figure-4**: Bax/Bcl-2 ratio of proteins expression as evaluated by immunocytochemical reactivity of treated MCF-7 cells and controls, for 24 hours. A significant difference at (*P*< 0.05) with respect to untreated group. Data represent means of three different experiments; bars, standard deviation and a: significant (*P* ≤ 0.05) with respect to the control (triplicate).**

Organoleptic or sensory quality analysis of food gruel:

Sensory analysis indicates a significant difference in aroma, texture and general acceptability.The Moringa-(2.5%) sample (6.33 ± 1.41) and the control sample (8.44 ± 0.72) had significantly higher ratings ($p \leq 0.05$) for all organoleptic tests than the other Moringa samples **(Figure 5)**.

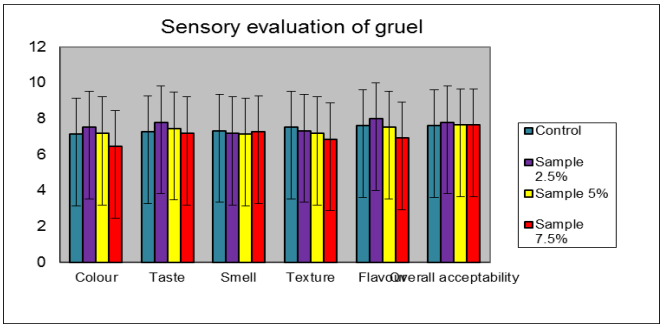

Figure-5: Comparison between the different organoleptic quality parameters (Sensory evaluation) of gruel with different levels of DMOL.

Organoleptic or sensory quality analysis of biscuits:

In acceptability test, Hedonic scale showed that the sample-1(2.5%) biscuit was more acceptable comparing with all quality characteristics by the judge **(Figure 6).**

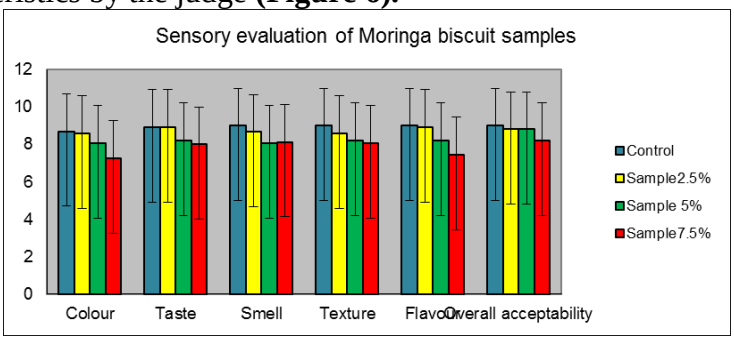

Figure-6: Comparison between the different organoleptic quality parameters (Sensory evaluation) of biscuits with different levels of DML.

Chemical analysis of dry *Moringa oleifera* leaves:

The quanitative proximate composition of whole leaf of *Moringa oleifera* in g/100g showed presence of all the nutrients tested (**Table 3**) while the quantitative analysis result was presented as moisture (8.71), protein (23.27), carbohydrate (35. 34), fats (2.91), fiber (22.0) and ash (7.78).

Table3: Nutritive values of dry *Moringa oleifera* leaves

samples	Moisture	protein	Carbohydrates	Fats	Fiber	Ash
DMOL	8.7	23.27	35.34	2.91	22.0	7.78

The quanitative analysis of minerals composition of *Moringa oleifera* leaf in mg/100g showed presence of all the tested minerals as iron (26.52), calcium (44.23), zinc (7.51), magnesium (147), copper (0.6), potassium (337), Manganese (0.36), Sodium (9), and phosphorous (112) mg/100g respectively (**Table 4**).

Table 4: Minerals composition of dry *Moringa oleifera* Leaves

parameter	Fe mg/100g	Ca mg/100g	Zn mg/100g	Mg mg/100g	Cu µg/100 g	K mg/100g	Mn mg/100g	Na mg/100g	P mg/100g
DMOL	26.52	44.233	7.51	147	0.6	337	0.36	9	112

Chemical analysis of gruel food:

Nutrient contents of normal weaning food that was estimated by using different analytical methods per 100 gm (normal weaning food) of product contain 21.1% moisture, 6.1% protein, 4.7% fat, 66.1% carbohydrate and 2.0% ash respectively. But after incorporating of moringa leaves, the nutritive values were improved **(Figure 7).**

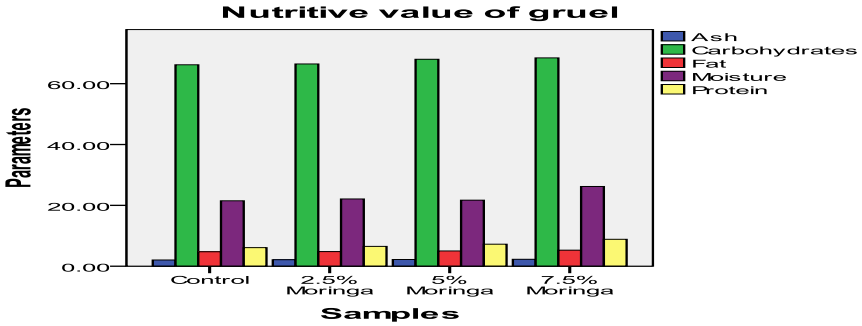

Figure-7: Nutritive values of different gruel samples with DML

Chemical analysis of biscuits samples

The proximate composition of the sample-4 biscuits contained 7.63% as moisture, 10.95 % (protein), 6.38 % (fat), 73.42 % (carbohydrate), 1.94% (ash) respectively. Above nutritional quality assessment concludes that Sample-4 biscuit is better than other samples. There was a significant increase in the iron content of biscuits with the incorporation of DML (dry Moringa leaves). The iron content increased from 5.65 to 7.73 mg with an increase in DML from 0 to 10%. The

calcium content of control biscuit was 11.42 mg % and biscuit with 10% DML were 14.25 mg /100g respectively. The significant increase in the calcium content is due to the presence of higher calcium content in DML **(Figure 8)**.

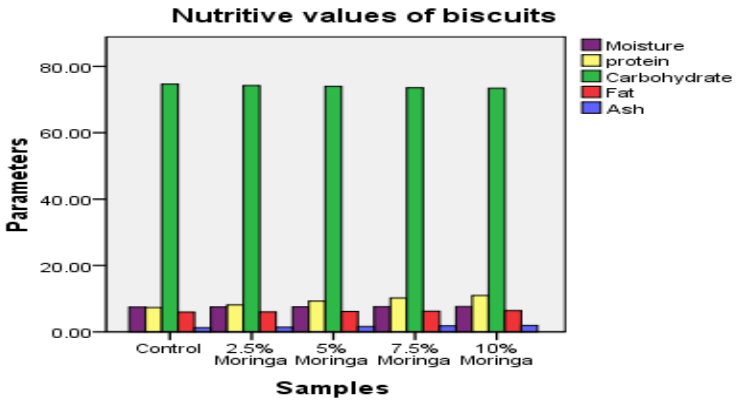

Figure -8: Nutritive values of different biscuit samples with DMOL

Discussion

In this study, MoWE was examined for its potential as an anticancer drug candidate on MCF-7. Preliminary studies on some of the medicinal plants have indicated their cytotoxicity towards pathogenic bacteria and cancer cell lines (**Mensah *et al*., 2006 and Bayor *et al*., 2007**).They examined some traditional medicinal plants and identified their cytotoxicity towards MCF-7 cells and other cancer cell lines.

This study evaluated the anticancer potential of *Moringa oleifera* water extract on MCF-7 cells. Results revealed moderate cytotoxic effect of MoWE on tested cells in parallel with **Aliyu *et al*., (2014)**. *Moringa oleifera* water extract exerted cytotoxic potential towards MCF-7. The established phytochemicals isolated from the medicinal plants could also be responsible for the observed cytotoxicity (**Olugbade *et al*., 2000; Umukoro & Ashorobi, 2007 and Ukeh *et al*., 2009**). Such phytochemicals have been reported to induce cytotoxic activity on other cancer cell lines (**Heo *et al*., 2004**).

Apoptosis has been employed to study the mechanism of action of bioactive compounds (**Koopman *et al*., 1994 and Xing *et al*., 2011**). The process involves a change in refractive index of the cell followed by cytoplasmic shrinkage and nuclear condensation, blebbing of the cell membrane and formation of "apoptotic bodies" (**Kerr *et al*., 1972 and**

Hengartner, 1997). The potency of *Moringa oleifera* to induce apoptosis in cancer cells largely depend on antioxidant capacities of its phytochemical constituents which are majorly natural phenolic compounds (**Rushworth & Micheau, 2009 and Lu *et al*., 2013**). These antioxidants (phenols) are chemicals capable of scavenging free radicals or stabilize free reactive oxygen species to prevent oxidative stress within the cell environment (**Dai Lu and Mumper, 2010**). However, only a few studies have reported the anticancer activity of *Moringa oleifera* leaves, and most of them have focused on the evaluation of their efficacy with respect to tumor suppressive activity, but not on the molecular basis of the tumor suppressive activity.

In this study, MoWE induced apoptosis through intrinsic mitochondria pathway as it induced up-regulation of Bax and the parallel down-regulation of Bcl-2 expression. Moreover, the nuclear condensation and abnormal morphology were noticed in treated cells using Giemsa stain. These anticancer activities of MoWE can be attributed to its antioxidant properties proved by DPPH scavenging activity in this study (**El-Nabi *et al*., 2018**). Furthermore, the investigated contents of polyphenolics, flavonoids and tannins also support the antioxidant and anticancer properties of the extract (**Tohamy *et al*., 2016**). Hence, the MoWE can be involved as a food supplement to achieve the anticancer properties after further advanced investigations and trials.

During the production of ogi (gruel), lose nutrients, including protein and minerals from the grain during the sieving. Ogi has been shown to be of low nutritional quality (**Akinrele and Bassir, 1967 and Abioye and Aka, 2015**). The addition of MOLP or *Moringa oleifera* Flower Powder (MOFP) to ogi was found to improve the nutritional value of maize or millet porridge (**Arise *et al*., 2014 and Abioye and Aka, 2015**). Incorporation of powdered *Moringe oleifera* leaves into baby foods can lead to the diversity of eating, ensuring food security and reducing some micronutrient deficiency diseases (**Odinakachukwu *et al*., 2014**). Supplementary foods containing *Moringa oleifera* powder either as part of an integrated diet of leguminous beans (MCL-35 g) or when sprayed as a dietary supplement (MS-5G) on normal baby foods were acceptable (**Boateng *et al*., 2017**). Extracts from *Moringa oleifera* leaf showed the presence of all phytochemicals (flavonoids, anthraquinone, alkali, saponin, steroids, terpenoids, heart glycosides, anthocyanins, tannins, and carotenoids) with many phytochemicals

(**Onyekwere and Felix 2014**). Other nutrients, such as protein, calcium, iron, and phosphorus, increased significantly after the addition of MOLP. The effect of MOLP on nutrient content and functional properties of Ogi was found to differ between authors. In this study, dry *Moringa oleifera* leaves were added to cereals, chemical analysis of gruel which fortified by DML showed that higher nutritive values than the control.

Several attempts have been made by researchers either to reduce or replace the amount of wheat flour used in the pastries completely drafting. According to **Gallagher *et al*. (2004)**, the functions of wheat gluten in the gluten-free dough were replaced by formulation such as bread is a major challenge for food scientists. Flour is widely commercially fortified with an extensive range of micronutrients added at varying levels (**Pachasn, 2018**). WHO provides guidelines on the fortification of flour with iron, folic acid, vitamin B12, vitamin A and zinc (**WHO, 2009**). In many countries, flour is enriched with B vitamins to compensate for micronutrient losses during the flour milling process. A dry powder premix of micronutrients is added after the grinding step and mixed with flour to give fortified flour (**Johnson and Wesley, 2010**). According to **Claughton and Pearce (1989)**, baked snacks such as cookies are widely consumed in many parts of the world. It is used for nutrition and nutrition improvement programs, especially among low-income groups. *Moringa oleifera* seed (**Ogunsina *et al*., 2010**) or leaf (**Dachana *et al*., 2010; Kar *et al*., 2013; Manaois *et al*., 2013 and Alam *et al*., 2014**) was also added to wheat biscuits or cake fortification. Protein content increased by adding 10% and 20% of MOSF to 45% and 90%, respectively. The addition of 10% of the Moringa flower powder to the wheat cookies resulted in a 45% increase in protein content than those mentioned in 10% of the MOLP-supported cookies by different authors (approximate 1% increase) (**Alam *et al*., 2014**), approximately 22% increase (**Dachana *et al*., 2010**). In this study, increased addition of DML from 0 to 10% showed that the sensory evaluation biscuit incorporated with 2.5% DML was acceptable. Above the 5% level adversely affected the quality of biscuits. The addition of 10 % DML significantly increased the protein, iron, and calcium. 10 % DML increased the nutritional value of biscuit but rejected in sensory evaluation. So, this study was concluded that the DML biscuits have the potential to serve as valuable sources of protein, iron, and calcium**.**

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دراسة التأثيرات المضادة للسرطان للمستخلص المائى لأوراق نبات المورينجا أوليفيرا على خلايا سرطان الثدي MCF-7 مع إمكانات استخدام مسحوق أوراقها كإضافة في بعض المنتجات الغذائية

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الملخص العربي

يعد سرطان الثدي أكثر أنواع السرطان شيوعًا بين النساء ، حيث يمثل ٢٣٪ من إجمالي الحالات الجديدة والسبب الرئيسي الثاني لوفيات السرطان لدى النساء. لقد أثبتت الأدوية الطبيعية أنها مصدر جيد للعناصر الفعالة في الادوية ذات القدرة الصيدلانية. يحتوي نبات المورينجا أوليفيرا على مواد كيميائية نباتية متنوعة تظهر نشاطًا مضادًا للسرطان من خلال التأثيرات السامة على خلايا سرطانية مختلفة. تتمثل أهداف هذه الدراسة في استكشاف آثار المركبات الطبيعية للمستخلص المائي لأوراق نبات المورينجا أوليفيرا (MoWE) على تكاثر خلايا MCF-7. و قد أظهرت النتائج أن MoWE يمتلك نشاط DPPH المضاد للأكسدة (٧٤,٥٣٪). بلغ إجمالي الفينولات الكلية والفلافونويدات والتانينات ٤٤,٧٧ ملليجرام من GAE / مل ، ٥,٨٦ و ٢٢,١٦ ملليجرام QE / مل ، على التوالي. كما اظهرت MoWE أيضًا تأثيرًا سامًا للخلايا على خلايا MCF-7 عند تركيز IC50 = 600µg / ml. تم تسجيل ارتفاع في نسبة Bax / Bcl-2 أربعة عشر مرة في المجموعات التي تمت معالجتها بواسطة MoWE. هذه النتائج تشير إلى أن للمستخلص المائي لأوراق نبات المورينجا أوليفيرا قد يكون له تأثير مفيد للحد من نمو سرطان الثدي ، وهذا يعتبر استراتيجية علاجية جديدة لعلاج السرطانات البشرية. الهدف الثاني هو دراسة إمكانية استخدام نبات المورينجا اوليفيرا (الورقة الكاملة) كمضافات غذائية طبيعية في بعض منتجات الاغذية خاصة للاطفال لحمايتهم مستقبلا من الاصابة بالسرطان.