

ANTI-PROLIFERATIVE EFFECT OF *Aerva javanica* EXTRACTS ON MCF7 AND MDA-MB-231 BREAST CANCER CELL LINES

(Kesan Anti-proliferasi Ekstrak *Aerva Javanica* Ke Atas Titisan Sel Kanser Payudara MCF7 dan MDA-MB-231)

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Abstract

In this study, the medicinal plant *Aerva javanica* was sequentially extracted with n-hexane, chloroform and 80% methanol using maceration technique. The anti-proliferative effect of the three extracts was assessed on two different breast cancer cell lines, MDA-MB-231 and MCF7, using MTT assay. 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) scavenging activity assay was used to assess the antioxidant activity of the three extracts. The result showed that the chloroform extract exerted an inhibitory effect in a concentration dependent manner on both cancer cell lines with IC₅₀ values of 32.7 ± 0.7 µg/ml and 40.9 ± 2 µg/ml on MDA-MB-231 and MCF7 respectively. 80% methanol extract exhibited cytotoxic effect on MCF7 with IC₅₀ value of 96.6 ± 2.9 µg/ml, whereas had no cytotoxic effect on MDA-MB-231 cell line (IC₅₀ > 200 µg/ml). n-hexane extract found to be less cytotoxic against both cancer cell lines with IC₅₀ values of 178.3 ± 3.7 and 196.9 ± 2.8 µg/ml on MDA-MB-231 and MCF7, respectively. The highest IC₅₀ value for scavenging activity was shown by 80% methanol extract (46.5 ± 2.2 µg/ml) followed by chloroform extract (78.3 ± 3.6 µg/ml). On the other hand, the extract prepared in n-hexane showed no scavenging activity effect (IC₅₀ > 1000 µg/ml). *Aerva javanica* possesses moderate anti-proliferative activity against human breast cancer cell lines with some cytoselectivity towards MDA-MB-231 compare to MCF7 cells. Further studies are needed to identify the potential anticancer component (s) from *Aerva javanica*.

Keywords: MTT assay, *Aerva javanica*, MCF7, MDA-MB-231

Abstrak

Dalam kajian ini, tumbuhan ubatan *Aerva javanica* telah diekstrak secara berturutan dengan n-heksana, kloroform dan 80% metanol menggunakan teknik pemaseratan. Kesan anti-proliferasi daripada tiga ekstrak dinilai pada dua titisan sel kanser payudara yang berbeza iaitu MDA-MB-231 dan MCF7, menggunakan ujian MTT. Ujian aktiviti hapus sisa DPPH telah digunakan untuk menilai aktiviti antioksidan daripada tiga ekstrak. Keputusan menunjukkan bahawa ekstrak kloroform memberikan kesan perencatan bergantung kepekatan ke atas kedua-dua jenis sel kanser dengan nilai-nilai IC₅₀ 32.7 ± 0.7 µg/ml dan 40.9 ± 2 µg/ml pada MDA-MB-231 dan MCF7 masing-masing. Ekstrak 80% metanol menunjukkan kesan sitotoksik ke atas MCF7 dengan nilai IC₅₀ 96.6 ± 2.9 µg/ml, tetapi tidak memberikan kesan sitotoksik ke atas MDA-MB-231 (IC₅₀ > 200 µg/ml). Ekstrak n-heksana didapati kurang sitotoksik terhadap kedua-dua titisan sel kanser dengan nilai-nilai IC₅₀ 178.3 ± 3.7 dan 196.9 ± 2.8 µg/ml masing-masing ke atas MDA-MB-231 dan MCF7. Aktiviti hapus sisa yang tertinggi ditunjukkan oleh ekstrak 80% metanol (IC₅₀ 46.5 ± 2.2 µg/ml) diikuti dengan ekstrak kloroform (IC₅₀ 78.3 ± 3.6 µg/ml). Berbeza dengan ekstrak n-heksana

yang tidak menunjukkan kesan aktiviti hapus sisa ($IC_{50} > 1000 \mu\text{g/ml}$). *Aerva javanica* mempunyai aktiviti anti-proliferatif sederhana terhadap titisan sel kanser payudara manusia dan lebih selektif terhadap sel-sel MDA-MB-231 berbanding MCF7. Kajian lanjut diperlukan untuk mengenalpasti komponen yang berpotensi sebagai anti-kanser daripada *Aerva javanica*.

Kata kunci: Ujian MTT, *Aerva javanica*, MCF7, MDA-MB-231

Introduction

Breast cancer is the most common type of cancer and the main cause of cancer death among women in both developed and developing countries. It accounts for 25% of all cancer diagnosed cases and 15% of all cancer female deaths [1]. New advances in research have produced medications for treating different stages of breast cancer. These chemically synthesized agents while being effective, often have some major side effects such as loss of appetite, muscle and joint pain, weakness, dizziness, vaginal bleeding, hair thinning and weight loss. The huge demand for herbal medicines is due to their wide biological activities, higher safety and lower costs [2]. Plants have shown an important role in the development of cancer therapy. Vincristine and vinblastine from *Catharanthus roseus* and paclitaxel from the bark of the Pacific yew tree, *Taxus brevifolia* are well known examples of plant-based anti-cancer drugs [3]. Investigating medicinal plants to explore traditional medicines could lead to the development of novel drugs.

Aerva javanica is a medicinal plant belongs to Amaranthaceae family [4]. It is a perennial herb extensively found in various regions of the tropical world. It is native to Africa, but can also be found in some Asian countries [5]. In Sudan, *A. javanica* plant or *Ras Elshaieb* as locally known has been used in traditional medicine in different parts of the country. In North Kordofan Province the plant is used for treating ulcers, wounds, abdominal pain and as anti-plasmodial. In the White Nile province, the plant is used against snake bites, breast cancer and rheumatism [6].

Phytochemical analysis studies of *A. javanica* revealed the presence of different important phytochemical constituents which include flavonoids, alkaloids, triterpenes anthraquinones, coumarins, diterpenes, triterpenes, sterol, saponins, carbohydrates, tannins and polyphenols [7, 8, 9].

In Sudan, *A. javanica*, is considered as an important traditional medicinal plant and has been used for treating various diseases, including cancer; however, its anti-cancer properties have not been investigated yet. The main objective of this study was to screen three *A. javanica* extracts for their cytotoxic effects on MCF7 (estrogen-receptor positive) and MDA-MB-231 (estrogen-receptor negative) breast cancer cell lines and to assess the antioxidant activities of the different extracts.

Materials and Methods

Reagents and chemicals

Dulbecco's modified eagle medium (DMEM), fetal bovine serum (FBS), penicillin/streptomycin and trypsin-EDTA were purchased from Gibco, USA. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) reagent, phosphate buffered saline tablets, dimethyl sulfoxide (DMSO) and 1,1-diphenyl-2-picryl-hydrazyl (DPPH) were obtained from Sigma-Aldrich, Germany.

Plant sample collection and identification

The whole plant of *Aerva javanica* was collected from Nile river banks, state of Khartoum, Sudan, in November 2015. The plant was authenticated by the plant taxonomist Yahya Suliman Mohamed and voucher specimen was deposited in the herbarium of Medicinal and Aromatic Plants Research Institute (MAPRI) – National Center for Research, Khartoum, Sudan.

Plant extraction

The whole plant was dried under shade for two weeks and then was grounded to make a fine powder. Powdered whole plant (500 g) was macerated sequentially with n-hexane, chloroform and 80% methanol for 3 days at 50 °C. The ratio of raw material to solvent was 1:10. The extracts were filtered through Whatmann No. 1 filter paper and concentrated using rotary evaporator at 50 °C, and then all the extracts were put in oven at 50 °C until complete dryness. The stock solutions of the extract were prepared at 10 mg/ml and kept at 4 °C until use.

Cell culture

Human breast adenocarcinoma cell lines MCF7 and MDA-MB-231 were originally obtained from American Type Culture Collection (ATCC, Rockville, MD, USA). MCF7 and MDA-MB-231 cells were grown in DMEM supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. All cell lines were incubated in a humidified incubator at 37 °C under an atmosphere containing 5% CO₂. The cells were sub cultured when nearly confluent using 0.25% trypsin-EDTA.

Cell viability assay

Cell viability and proliferation were assessed by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide) dye reduction assay. In brief, the cells were seeded in a 96-well plate (3×10^3 cell/well for MCF7 and 5×10^3 cell/well for MDA-MB-231) and treated with different concentrations of the extracts (6.25 – 200 µg/ml) for 48 hours. MTT solution (5 mg/ml in PBS) was added (20 µl/well) and incubated for 3 hours. The formazan crystals formed by the viable cells were dissolved by adding 200 µl/well DMSO. Optical density was measured at 570 nm wavelength (reference filter 630 nm) using an ELISA plate reader (TECAN, sunrise). Cell survival rates were expressed as the percentage of untreated controls, and the 50% inhibitory concentrations (IC₅₀) of the extracts were calculated.

1, 1-diphenyl-2-picryl-hydrazyl (DPPH) free radical analysis

1,1-diphenyl-2-picrylhydrazyl (DPPH) assay was carried out to evaluate the scavenging activity of the extracts. The stock solution of DPPH was prepared at a concentration of 200 µM in absolute methanol while stock solutions of the extracts were prepared at a concentration of 10 mg/ml. 100 µl of DPPH was dispensed in 96-well plate and immediately, 100 µl of samples were added at final concentrations of 12.5, 25, 50, 100, 200 and 400 µg/ml. Ascorbic acid was used as positive control. Methanol alone and methanol with DPPH were used as blank and negative control, respectively. The mixtures were incubated for 30 minutes in the dark and then the absorbance was measured at 517 nm using TECAN microplate reader (Tecan sunrise). The dose response curves were obtained and then used to calculate the median inhibitory concentration (IC₅₀). The results were expressed as mean ± SEM (n = 6).

Statistical analysis

All of the cytotoxic and antioxidant experiments in the study were carried out for at least three times in triplicate and results were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) and Dunnet's Multiple Comparison Test were used to assess IC₅₀ values and cytotoxicity data. P < 0.05 was considered as statistically significant.

Results and Discussion

Extraction

Extraction efficiencies (% yield) of the three solvents, n-hexane, chloroform and 80% methanol showed significant differences (P < 0.05). The maximum extraction yield was shown by 80% methanol (2.8 %) followed by n-hexane (1.1 %) and chloroform (0.6%). Figure 1 shows the extraction yields of the different solvents.

(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay

The three extracts exhibited variations in their cytotoxic effect on the two examined breast cancer cell lines with different IC₅₀ values. The chloroform extract showed the lowest IC₅₀ values of 32.7 ± 0.7 and 40.9 ± 2 µg/ml on MDA-MB-231 and MCF7, respectively. 80% methanol extract exhibited cytotoxic effect on MCF7 with IC₅₀ value of 96.6 ± 2.9 µg/ml, while had no cytotoxic effect on MDA-MB-231 cell line (IC₅₀ > 200 µg/ml). The n-hexane extract showed high IC₅₀ concentrations for both cancer cell lines (Table 1).

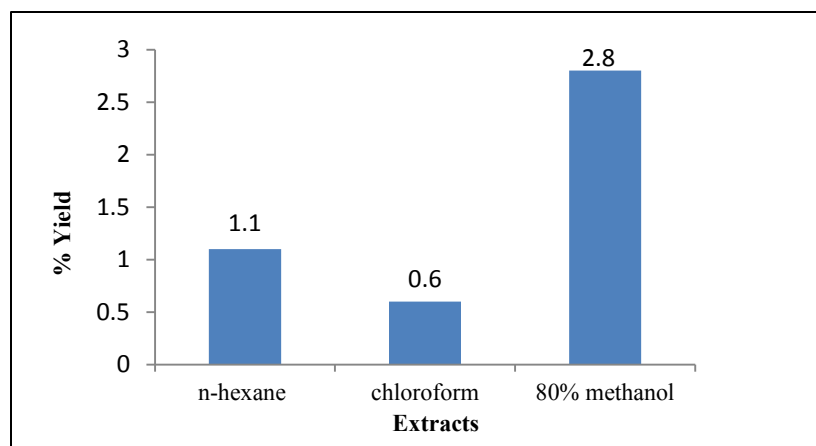


Figure 1. Extraction yields of *Aerva javanica* using the three different solvents

Table 1. IC₅₀ (μg/ml) values of the three extracts against the two cancer cell lines

Extracts	IC ₅₀ value (μg/ml)	
	MCF7	MDA-MB-231
n-Hexane	196.9 ± 2.8	178.3 ± 3.7
Chloroform	40.9 ± 2.3	32.7 ± 0.7
80% Methanol	96.6 ± 2.9	> 200

The results are presented as mean ± SEM. Each experiment was repeated three times; (n=3)

The chloroform extract exerted moderate cytotoxic activity in a concentration dependent manner on both cancer cell lines MDA-MB-231 and MCF7 cell line (Figure 2). Unexpectedly, the cytotoxic effect of the extract was more potent in the highly invasive breast cancer cell line; MDA-MB-231.

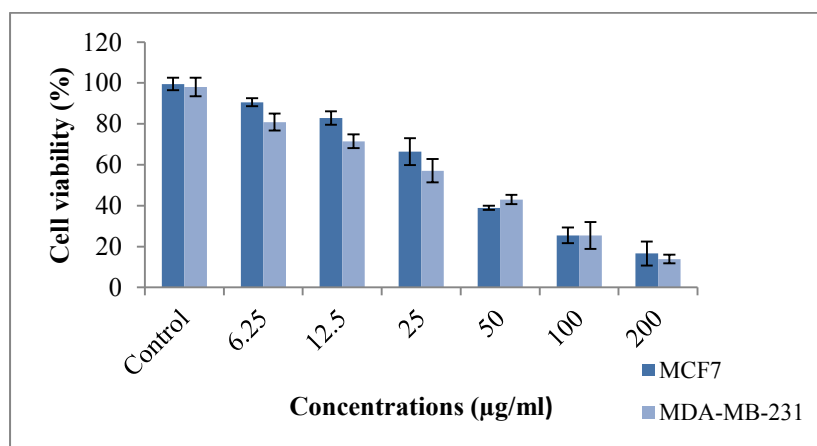
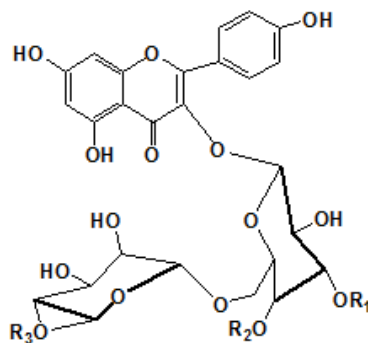


Figure 2. Effect of *A. javanica* chloroform extract on MDA-MB-231 and MCF7 cell viability. The cells were treated with different concentrations and DMSO as a control for 48 h and the cell viability was tested using MTT assay

The pharmacological effects of medicinal plants can be attributed to their main constituents such as alkaloids, flavonoids, coumarins, lignans, saponins and saccharides. Flavonoids are considered as an important phytochemical, with various anti-cancer activities. It has been reported that many flavonoids significantly decrease cancer cell viability via the upregulation of caspase activity [10]. The flavonols, kaempferols and kaempferol glycosides are found in a variety of plants and plant-derived foods [11, 12, 13]. Several reports have shown that kaempferol and/or some kaempferol glycosides induce cell death in a variety of cancer cells from different tissues, including human leukemia cell lines [14] and breast cancer cell lines [15 – 18]. Many flavonol glycosides have been isolated from *A. javanica* as major phytoconstituents [9].

Recently, three kaempferol glycosides have been isolated from *A. javanica* flowers, namely, kaempferol-3-O-b-D-[4000-E-p-coumaroyl-a-L-rhamnosyl(1→6)]-galactoside (1), kaempferol-3-O-b-D-[4000-E-p-coumaroyl-a-L-rhamnosyl(1→6)]-(300-E-p-coumaroyl)-galactoside (2), and kaempferol-3-O-b-D-[4000-E-p-coumaroyl-a-L-rhamnosyl(1→6)]-(400-E-p-coumaroyl)-galactoside (3) [19]. Figure 3 shows the structure of the three acylated flavonoid glycoside compounds isolated from *A. javanica*.



- 1: R1 = R2 = H, R3 = *E-p*-coumaroyl
 2: R1 = *E-p*-coumaroyl, R2 = H, R3 = *E-p*-coumaroyl
 3: R1 = H, R2 = R3 = *E-p*-coumaroyl

Figure 3. Structure of the three acylated flavonoid glycoside compounds isolated from *A. javanica*

Coumarins are an important medicinal phytochemicals present in different medicinal plants. Coumarins have a wide range of biological activities including anticancer properties [20]. Both natural and synthetic coumarins exhibited potent anticancer activity against various types of cancer, including breast cancer with low toxicity and good selectivity [20]. Figure 4 shows the coumarin structure. Previous study revealed the presence of coumarins in *A. javanica* chloroform fraction; however, no coumarin compounds have been isolated from the plant yet [8].

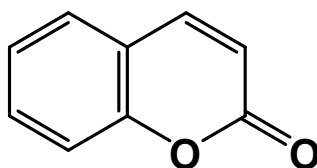


Figure 4. The structure of coumarin

The anti-proliferative effect exhibited by the chloroform extract in this study could be due to the presence of one or both of these important phytochemicals. Further studies are needed to identify the bioactive compounds that present in *A. javanica* chloroform extract and to elucidate the mechanism of action for these compounds. Our results showed that *A. javanica* chloroform extract was more cytotoxic to MDA-MB-231 than MCF7 cell line. The

differences in susceptibility of different tumor cell lines have been shown by several research groups [21, 22, 23]. The differential response of these two cancer cell lines could be due to the differences in their signaling pathway responsible for the cytotoxic effect [23]. It should be noted that the MDA-MB-231 cell line does not express the estrogen receptor [24] and the fact that the extract showed cytotoxic effect on this cell line indicates that estrogen-receptor signaling pathway is not required in its mode of action. This is very interesting since many conventional drugs that are used in the treatment of breast cancer, such as tamoxifen, raloxifene and toremifene require estrogen-receptor to exert their cytotoxic effect [25]. Furthermore, the response of estrogen-receptor negative subtype of breast cancer to conventional drugs is very poor [24, 26]. The possible molecular mechanisms underlying the differential responses of MCF-7 and MDA-MB-231 cells to the cytotoxic effects of *A. javanica* need to be further investigated.

Antioxidant activity

The results obtained from DPPH free radical scavenging activity assay in this study showed that the 80% methanol extract demonstrated the most potent antioxidant activity ($46.5 \pm 3 \mu\text{g/ml}$) followed by chloroform extract ($78.3 \pm 4 \mu\text{g/ml}$), whereas the extract prepared from n-hexane showed poor DPPH scavenging activity as the IC_{50} value estimated to be more than 1000 $\mu\text{g/ml}$ (Table 2).

Table 2. IC_{50} values of the three extracts of *Aerva javanica* evaluated for DPPH scavenging activity

Extract	DPPH ($\text{IC}_{50} \mu\text{g/ml}$)
n-Hexane	> 1000
Chloroform	78.3 ± 3.6
80% Methanol	46.5 ± 2.2
Ascorbic acid	3.3 ± 3.9

The 80% methanol extract contains significantly high free radical scavenging activity. Thus, it was found that free radical scavenging activity increased with the increase of the solvent polarity. This observation strongly supports previous results [27] as the methanol extract contains the highest amount of phenolic compounds. Phenolic compounds are known to be powerful antioxidants; they possess scavenging ability due to their hydroxyl groups [28]. So, in the present study, the antioxidant activity of the hydroalcoholic extract was possibly due to its high phenolic content.

Conclusion

The result of the present study concludes that *Aerva javanica* shows moderate cytotoxic effect on two different breast cancer cell lines with some cytoselectivity towards MDA-MB-231 compare to MCF7 breast cancer cell line. The plant also exerts moderate free radical scavenging activity. Further studies are needed to identify the potential anticancer component(s) from this medicinal plant and to determine the mechanism(s) of action underlying its cytotoxicity.

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