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Characterization of Local Okra Seed *(Abelmoschus Esculentus)* Extract and Evaluation of Bioactivity

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ABSTRACT

A. esculentus (okra) plant is an economically important medicinal plant grown in tropical and subtropical parts of the world. The fruit and seeds of the *A. esculentus* (okra) plant; which are rich in oil and protein. The *in vitro* cytotoxic, bactericidal, and antioxidant activities of extracts obtained from different parts of okra (leaves, fruits, and seeds) were studied widely in the medical industry. This study evaluated the biocompatible and antioxidant potential of an optimized ethanolic extract of *A. esculentus* seeds. Commercially obtained *A. esculentus* seeds were first extracted and characterized by UV-Vis and FT-IR spectrophotometry. *In vitro*, antioxidant activity of okra seed extracts was evaluated using 1,1 -diphenyl -2picrylhydrazyl (DPPH) with different concentrations (0.5-5 mg/mL). Results show that the antioxidant activity increased in direct proportion to the increase in the concentration of the extract. *In vitro*, the cytotoxic effect of the extracts on C6 Glioma cancer cell line and L929 mouse fibroblast cell line was studied using 3-(4, 5-dimethylthiazolyl -2,5-diphenyl-tetrazolium bromide (MTT) assay with the concentrations between 50-1000 µg/mL. MTT results showed effective cytotoxicity on cancer cell lines with increasing extract concentration (IC50 value for C6 was 273.4 µg/ml, while the IC50 value for L929 was 431.45 µg/ml). The study indicates that the extract isolated from *A. esculentus* seeds shows that all concentrations have a substantial amount of anticancer and antioxidant activity. In conclusion, *A. esculentus* seeds could be considered innovative products and be proposed for alternative end-uses in pharmaceutical industries with antimicrobial and bioactive properties.

Keywords: *Abelmoschus esculentus*, Cancer, C6 Glioma, L929, Cytotoxicity, Antioxidants.

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Introduction

Different civilizations have widely used functional plants for thousands of years for traditional treatment purposes. They are exploited for their bioactive compounds like polyphenols, carotene, folic acid, thiamine, vitamin C, and amino acids, which are highly demanded in cosmetic, pharmaceutical, and nutraceutical industries [1,2]. It is reported that plants have antimicrobial, anticarcinogenic, anti-glycemic, antioxidant, anti-cholesterol, antiparasitic, and anti-inflammatory properties, thanks to the phenolic compounds found at different levels in almost all parts of plants [3,4].

A. esculentus is a member of the mallow (Malvaceae) family and is often used as an edible immature fruit. This herbaceous annual plant is native to Africa (Ethiopia) and is now almost cosmopolitan, cultivated in tropical, subtropical, and warm temperate climates in different countries from Africa to Asia, southern Europe, and America [5,6]. In traditional medicine, okra (A. esculentus) is thought to exhibit diuretic, antispasmodic, relaxing, laxative, and anti-cancer effects. It is also known for its beneficial mucus content. The public uses it to treat diseases such as colds, gonorrhea, dental disease, and bronchitis. *A. esculentus* attracts the attention of the public and researchers. In addition to the fruit of the plant, its leaves, flowers, stems, and seeds are rich in oil and protein. In vivo and vitro antioxidant, antidiabetic, antihyperlipidemic, antimicrobial, antifatigue, hepatoprotective, and neuroprotective effects of okra (A. esculentus) have been reported [7-10]. A substance frequently mentioned in the okra seed extract is isoquercitrin, with higher bioavailability than quercetin, displaying a few chemoprotective effects against cancer [11]. Isoquercitrin has shown inhibition of urinary bladder and pancreatic cancer progress [12,13], as well as colon cancer suppression [14]. The profile of the bioactive components in different parts of *A. esculentus* (okra) is well documented: for its pod polyphenolic compounds, carotene, folic acid, thiamine, riboflavin, niacin, vitamin C, oxalic acid, and amino acids; for its seed polyphenolic compounds, mainly oligomeric catechins and flavonol derivatives, protein (i.e., high lysine levels), and oil fraction (in particular, its derived oil is rich in palmitic, oleic, and linoleic acids); for root carbohydrates and flavonol glycosides, and manly minerals, tannins, and flavonol glycosides for leaves.

Despite the numerous studies mentioned above, there is a paucity of reports regarding the anticancer effects of *A. esculentus* (okra) seeds. Extractive techniques and conditions used recently are almost obsolete and must be optimized for higher efficiency. Leonardo G. Monte and his colleagues researched the anti-tumor properties of a

newly discovered lectin from *A. esculentus* (AEL) on human breast cancer (MCF7) and skin fibroblast (CCD-1089SK) cells. Their results show that AEL in its natural form could potentially create an anti-tumor effect on breast cancer cells and could be a therapeutic support for the treatment of human breast cancer [15]. Watcharaphong et al. [16] investigated the effects of okra seed extract (OSE) on the proliferation and viability of three human cancer cell lines (MCF-7, HeLa, and HepG2). It was found that both the naturally applied extract and the extract loaded into polymeric micelles had dose-dependent cytotoxic effects on all three cell lines. OSE-loaded micelles were more cytotoxic than natural OSE, especially in the HepG2 cell line. Additionally, it was found that OSE inhibited the migration of MCF-7 and HeLa cells. Hayaza and coworkers [17] conducted a study to examine raw polysaccharides extract of okra (*A. esculentus* L.) as an anticancer agent against Huh7it human liver cancer cells. Their extract could reduce the metabolism of cancer cells. It also has potential as an anti-cancer agent by inhibiting up to 15 % cell growth at the highest dose.

Glioblastoma multiforme (GBM) stands as one of the most formidable challenges in oncology, characterized by its aggressive infiltration into surrounding brain tissue, resistance to conventional therapies, and high rates of recurrence [18]. Despite decades of research and therapeutic advancements, the prognosis for GBM patients remains bleak, with a median survival of only 12-15 months post-diagnosis. This pressing clinical need underscores the imperative for novel therapeutic approaches that can effectively target GBM with improved efficacy and reduced toxicity [19]. C6 is a glial cell line isolated from the brain of a rat with Glioma. C6 glioma cells are frequently used for glioblastoma research since they have high mitotic activity and were determined to be the cell line most similar to the mechanism of human brain tumors. L929 cells play a pivotal role in biological science with versatile applications. They serve as a crucial resource for toxicity assessment, aiding in evaluating the potential adverse effects of various compounds. The cytotoxic and anticancerogenic potentials of *A. Esculentus* seed extracts were determined for C6 rat glial cell and L929 rat fibroblastoma cell line using MTT at different concentrations.

A lot of research has been done on the antioxidants, anti-inflammatory, and anticancer activity of medical plants that are rich in polyphenols and flavonoids and are still being done [20,21]. While the interest of the biological activities of okra seed extract has not yet been explored for comparatively studied for cancer and normal cell lines. In addition, the importance of okra as a low-cost functional feed still deserves to be further highlighted in the potential of cancer treatment. Considering this, our study comprehensively evaluated the antioxidant properties and cytotoxic effects of *A. esculentus* seeds extract against C6 glioblastoma and L929 fibroblastoma cell lines using an in vitro model. The extractive techniques and conditions used in the present study are also optimized for higher efficiency.

Material and Method

Preparation of A. Esculentus Seed Extract

A. esculentus seeds were obtained from traditional markets in Turkiye. Then, they were washed thoroughly with distilled water to remove foreign material. To get the extract, briefly, the dried 6 g. of fresh *A. esculentus* seeds were rinsed with 30 mL of 70% ethanol in a shaking water bath (N-Biotek NB-30330) for 48 hours. The extracts were prepared using ethanol (70%) (ISOLAB), known as a good solvent for polyphenol extraction, safe for human consumption, low cost, and environmentally friendly in nature [22]. After 72 hours, the ethanol-soluble fraction was filtered through a 0.45 µm microporous membrane and concentrated in a vacuum oven (Nuve CO2 Incubator) at +50°C. It was then freeze-dried with a lyophilizer and stored in the refrigerator at +4°C until use.

Sigma-Aldrich provided suitable media and analytical-grade chemicals. All the chemicals were analytical grades and used without further modification or purification. Double-distilled and deionized water was used in the extraction and for rinsing the clusters.

Characterization of A. esculentus Seed Extract

A. esculentus extracts were prepared at a concentration of 2 mg/mL. In UV-Vis Spectrophotometry (Shimadzu UV-1700), the spectrums of each sample were used in the range of 200-800 nm by using a blank instead of pure water. At the same time, to analyze the chemical structure of the extract and the functional groups it contains, it was examined on an FT-IR spectrophotometer (Shimadzu Prestige 2100) in ATR mode, and FT-IR spectra ranging from 600 to 4000 cm-1 were used.

Antioxidant Activity Analysis of A. esculentus Seed Extract

The antioxidant activity of *A. esculentus* seed extracts were tested using a 1,1-diphenyl-2-picryl hydroxyl (DPPH) technique. To make the stock solution, 0.63 milligrams of DPPH were dissolved in 15.98 mL of ethanol. Filtration of the DPPH stock solution using methanol yielded a usable 0.1 mM concentration DPPH mixture with an absorbance of around 0.973 at 517 nm. To ensure the reliability of the test, the study was conducted with 3 repetitions.

In a 96-well microplate, 100 µL of DPPH stock solutions were combined with the concentration of *A. esculentus* seed extracts between 0.5 -5 mg/mL. As for negative control, 100 µL of DPPH solution and 100 µL of pure water were added to the control wells. After that, the plate was kept in complete darkness for 30 min. The absorbance was therefore determined at 517 nm. The following formula was used to compute the percentage of antioxidants.

% of antioxidant activity= [(Ac−As) ÷Ac] × 100

where: Ac—Control reaction absorbance; As—Testing specimen absorbance.

The absorbance and % antioxidant activity values of seed extracts were obtained with SkanIt™ Software for Microplate Readers using the Elisa Plate Reader (Thermo

Scientific Multiskan Go). Antioxidant activity values of the extracts were calculated using the Microsoft Excel program.

Cell Culture

The ethanolic plant extracts were screened for cytotoxicity using C6 glioma (glioblastoma multiforme - GBM) and L929 fibroblastoma (mouse fibroblast) cell lines. The C6 and L929 cell lines were seeded into 25 cm^2 plastic tissue culture flasks in DMEM/F12 medium (Sigma), supplemented with 10% FCS, 1% penicillin/streptomycin into 500 ml of DMEM/F12 medium containing L-glutamine. Cells were grown at 25 °C in a humidified atmosphere (85% humidity). After achieving exponential growth (approximately 90% confluent), cells were detached from culture flasks by brief exposure to trypsin (0.25% in PBS, pH 7.2–7.4), according to the standard trypsinization methods. The detached cells were collected by centrifugation (Nuve NF 800, 1000 rpm, 5 min, 25 °C) and counted on Thoma slides.

MTT Assay

The cytotoxicity analyses of *A. esculentus* seed powders at different concentrations on C6 Glioma and L929 fibroblastoma cell lines were performed comparatively by 3-(4,5-dimethyl thiazolyl- 2-yl)- 2, 5-diphenyltetrazolium bromide (MTT) assay, (ISO B. 10993-5: biological evaluation of medical devices). Briefly, intact C6 and L929 cells were seeded into 96-well plates at a $1x10^4$ cells/ml ratio in DMEM containing 5% fetal bovine serum (FBS) and 1% penicillin-streptomycin. Cells were incubated at 37°C in a 5% CO2 for 24 h. After the overnight incubation, the medium was replaced with a fresh medium containing a series of dilutions of increasing the *A. esculentus* seed powder concentrations applied to C6 and L929 cells from 50 µg/mL to 1000 µg/mL. C6 and L929 cells were seeded on wells without extracts as a control group. After 24 incubations, the medium from each well was replaced with an MTT reagent then plates were incubated at 37 °C for 3 h. Following incubation, formazan crystals formed in the cell were dissolved in DMSO, and absorbance was recorded at 570 nm with a 96-well plate reader. Results were obtained using SkanIt™ Software for Microplate Readers using the Elisa Plate Reader (Thermo Scientific Multiskan Go). Values are displayed as mean ± SD of a minimum of three times. Statistical analyses and IC50 values of the extracts were evaluated in GraphPad Prism 8 software to find the percent cell viability [23].

Results and Discussion

UV-VIS Spectrophotometer Results

The flavonoid isolate compounds were identified using a UV-Vis spectrophotometer to determine the absorbance value of the compound at the maximum wavelength. The UV-Vis spectrum of the *A. esculentus* extracts can be seen in Figure 1. The extracts caused a peak at a wavelength of 272 nm. It is known that tannins and phenolic compounds peak in the 270-280 nm range [24]. The spectrum results obtained were the maximum wavelengths that may occur due to the absorption of the benzoyl ring on the flavonoid structure, mainly occurring at a wavelength of 256 nm [25].

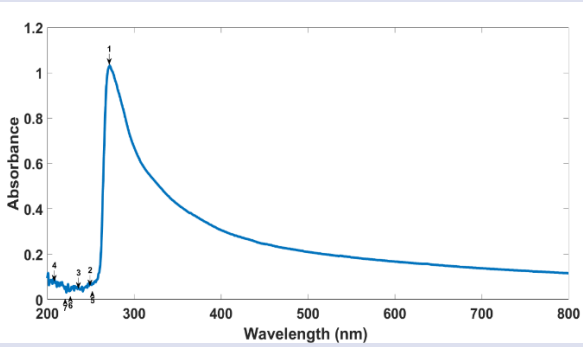

Figure 1. UV-Vis spectrophotometry of *A. esculentus* seed extract

FT-IR Spectrophotometer Results

The FTIR results shown in Figure 2 indicate the presence of various functional groups such as alkyl, ketone, aldehyde, carboxylic acids, esters, and amide in the aqueous and methanolic extracts of A. esculentus , respectively [26]. The FTIR confirmed that the so-called fingerprint region of the spectrum consists of characteristic bands between 1500 cm^{-1} and 1000 cm^{-1}, which can be attributed to polysaccharides. The band at 925 cm^{-1} belongs to the C-O stretching vibrations of alcohols/phenols [5]. The band located at 1041 cm^{-1} might refer to the C=O stretching vibrations of carbonyl compounds. Absorption at 1590 cm^{-1} due to ester carbonyl is detected and agrees with the okra spectrum of Archana et al. [27]. The band at 1402 cm^{-1} belongs to the stretching vibrations of aromatic C=C bonds. The band at 3285 cm^{-1} belongs to the hydrogen-bonded O-H stretching vibration due to water and carbohydrates [28]. The bands at 2851cm^{-1} belong to aliphatic compounds' asymmetric C-H stretching vibrations [29]. The bands at 552 cm^{-1}, 563 cm^{-1} and 591 cm^{-1} may be attributed to the C-O-H bending vibrations of aliphatic compounds or phenolic ring torsions [30,31]. Results agreed with those reported in the literature [32].

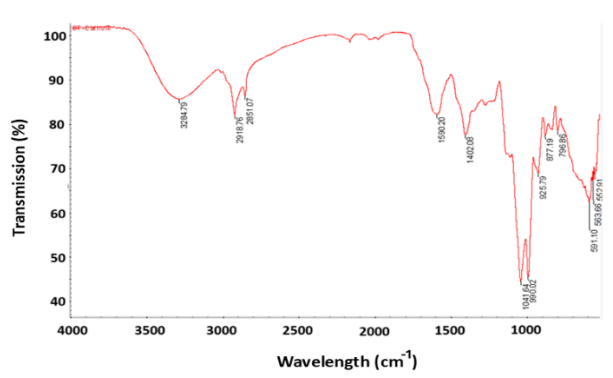

Figure 2. FT-IR spectrophotometry of *A. esculentus* seed extracts

Antioxidant Activity

The total antioxidant activity, measured by the DPPH method, of *A. esculentus* seed extracts was determined in a range of concentrations from 0.5 to 5 mg/mL (with an increase of 0.5 mg/mL). Antioxidants' ability to donate hydrogen or their radical scavenging impact on DPPH radical's scavenging activity. When a DPPH solution is combined with a material that can donate a hydrogen atom, diphenyl picryl hydrazine is produced in its reduced form, losing its violet color [33].

Table 1 and Figure 3 give the absorbance and % antioxidant activity values of seed extracts. The results showed that ethanolic *A. esculentus* extracts at different concentrations have remarkable antioxidant activities with concentration (dose) dependency.

Tablo 1. Absorbance and % antioxidant activity values of *A. esculentus* extract

Concentration (mg/mL)	Extract+DPPH	Extract + Ethanol	Difference	Antioxidant Activity (%)
Control	0,188	-	-	-
0,5	0,156	0,047	0,108	42,38
1	0,127	0,056	0,070	62,59
2	0,1185	0,058	0,0605	67,81
2,5	0,125	0,067	0,058	69,14
3	0,125	0,073	0,052	72,34
3,5	0,136	0,089	0,047	75,00
4	0,124	0,088	0,035	81,21
4,5	0,121	0,102	0,018	90,25
5	0,141	0,129	0,012	93,62

The results indicated a significant increase in the antioxidant activity at the highest concentration tested (93.62% at 5 mg/mL) compared to the lowest concentration (42.38% at 0.5 5 mg/mL). The antioxidant capacity of the extract should be related to its high phenolic content since polyphenols have been reported to possess antioxidant capacities**Hata! Başvuru kaynağı bulunamadı.**. The content of flavonoids in a plant extract could also result in higher antioxidant activity**Hata! Başvuru kaynağı bulunamadı.**.

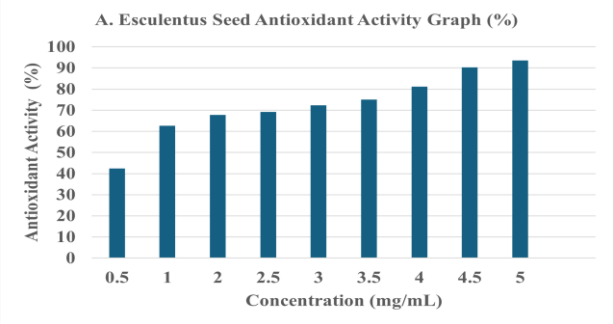

Figure 3. Antioxidant activity of *A. Esculentus* seed extracts

Cytotoxicity Results

The possible cytotoxic effects of ethanolic extracts were evaluated on L929 and C6 cells after 24 h of incubation with different concentrations of extracts ranging from 50 µg/mL to 1000 µg/mL (50, 100, 250, 500, 1000 µg/mL), comparing the results with the control group, which consisted of cells incubated only with the medium.

The results showed that the cytotoxic effect of okra extracts depended on the extract dose and the cell line in question. As it is seen in Figure 4, Abelmoschus Esculentus seed extracts produced a significant reduction in the viability of the L929 cell line compared to the control samples at all concentrations. It was observed that cell vitality decreased from 86.1% to 10.2% by increasing the *A. esculentus* concentrations applied to L929 cells from 50 µg/mL to 1000 µg/mL. The IC_{50} value for L929 cells was 431.45 µg/ml. Indeed, the determination of IC_{50} was not achieved concerning the incubation times tested. Thus, the extract seemed to be toxic, dependent on the concentration range **Hata! Başvuru kaynağı bulunamadı.**.

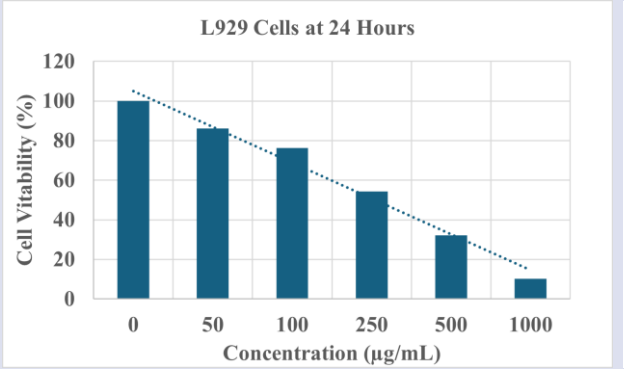

Figure 4. Cytotoxic activity (expressed as the percentage of viable cells) on L929 cell lines after 24 h of incubation with of *A. esculentus* seed extracts

When *A. esculentus* extracts were tested on the C6 Glioma cancer cell line, we observed a potentiated cytotoxic effect for the extracts, resulting in 72.19% C6 cell viability even at a lower concentration (50 µg/mL) than the control group. Figure 5 shows the cytotoxicity of *A. esculentus* extracts on C6 Glioma cells at the 24th hour. It was observed that by increasing the *A. esculentus* concentrations applied to C6 Glioma cells from 50 µg/mL to 1000 µg/mL, the vitality rate in the cells decreased from 72.2% to 12.9%. The IC_{50} value for C6 glioma cells was 273.4 µg/ml. The sample concentrations of 1000µg/ml, 500 µg/ml, 250µg/ml, 100µg/ml, and 50µg/ml show %12.9, %15.4, %28.2, %52.5, and %72.2 cell viability values against the glioma cancer C6 cell line respectively. The cytotoxic effect observed depended only on concentration, not the time on both cell lines. *A. esculentus* extracts at different doses (50 to 1000 µg/mL) showed lower toxicity on normal L929 cells than the cancer C6 cells. The toxicity of extract on cancer cells may be due to the high metabolic rate of cells. Hence, cancer cells have a greater possibility of absorbing *A. esculentus* extracts than normal cells. There are additional reasons why cancer cell lines may have lower IC_{50} values than normal cell lines, including strong extract interaction and

a high rate of cell division **Hata! Başvuru kaynağı bulunamadı.**. The results are consistent with numerous studies that found that flavonoid substances may induce cancer cell apoptosis [38–39].

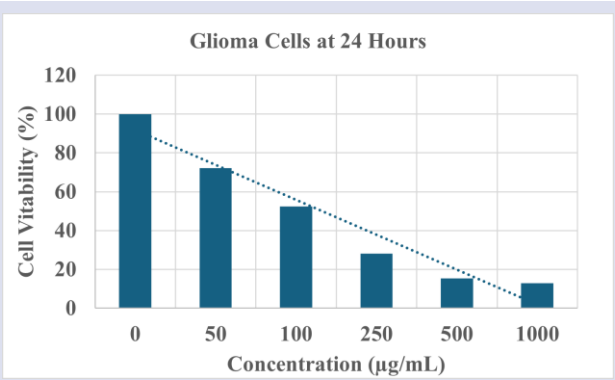

Figure 5. Cytotoxic activity (expressed as the percentage of viable cells) on C6 cell lines after 24 h of incubation with of *A. esculentus* seed extracts

Conclusion

The study indicates that the extract isolated from *A. esculentus* seeds has substantial anticancer and antioxidant activity at all concentrations. The toxicity results of extract at different doses (50 to 1000 µg/mL) showed on L929 cells lower than cancer cells. Due to the metabolic rate of cells, cancer cells may have a greater possibility of absorbing *A. esculentus* extracts than normal cells. The anticancer effect has been observed dose-dependent. The biological properties might be due to the synergistic actions of bioactive compounds such as a considerable amount of total phenolics and flavonoids. Many previous works have reported a highly significant relationship between total phenol content and antioxidant activity. The seed extract's antioxidant potential may be due to the presence of vitamins such as vitamin E. So, it could be concluded that the extract can act as a source of anticancer drugs and can be used to improve health status. There should also be a dependency on the dose use of ethanol, which offers the highest total phenolic contents. However, more research is needed to understand and implement these findings into clinical practice.

Further studies should also be conducted to evaluate the *A. esculentus* seed extract, which needs to be tested in animal models and clinical studies to understand better its effectiveness and possible side effects in cancer treatment. Additionally, researchers showed that the possibility of green synthesized nanoparticles with the help of *A. esculentus* can potentially act as anticancer and antibacterial agents. Therefore, scientists and healthcare professionals must continue to support studies and discoveries in this field. Investigating the potential of plant extracts in cancer treatment may contribute to developing more effective and natural treatment methods.

Conflicts of interest

The authors declare no conflict of interest.

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