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Research Article

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Abstract

Aerva javanica (Burm.f.) Juss. ex Schult. (Amaranthaceae family) has many pharmaceutical properties. This study aimed to determine the anticancerous effect of *A. javanica* methanol extract (AJME) on breast cancer cell lines and prostate cancer cell lines. The antibacterial potency of *A. javanica* solvent extracts was tested against *Micrococcus luteus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Shigella flexneri*. A screening of five concentrations of *A. javanica* was done on prostate and breast cancer cell lines using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay. The results showed that AJME had various levels of cytotoxicity toward both cancer cell lines. A significant decrease in the rate of cancer cells was associated with a higher concentration of the plant extract. The half maximal inhibitory concentration IC50 value was 4.50 μg/ml for the breast cancer cells and 14.51 μg/ml for the prostate cancer cells. The dried and fresh solvent extracts made with methanol and chloroform demonstrated maximum potency against all the tested pathogenic microbes. The petroleum ether and acetone extracts showed moderate activity, while the diethyl ether and hot water extracts had the lowest antibacterial activity. The gas chromatography-mass spectroscopy analysis showed that AJME had various chemical compounds that have many biological benefits. In conclusion, *A. javanica* is a promising candidate as a natural herb to treat cancers, more so in breast cancer than in prostate cancer, and it has potential as an antimicrobial agent against multidrug-resistant microbes.

Keywords

Aerva javanica, breast cancer, prostate cancer, organic solvent extracts, anticancer, antibacterial, phytochemicals

Introduction

Every year, millions of women around the world are diagnosed with breast cancer (Jemal et al., 2011). About 112.6 per 100,000 men are diagnosed with prostate cancer every year (Cremers et al., 2010). These data are based on the number of prostate cancer cases and deaths caused by that cancer between 2011 and 2015 in about 19.5 per 100,000 men per year (National Cancer Institute, https://seer.cancer.gov/). Current pharmaceutical drugs can only suppress tumor growth to a certain extent for all types of cancers. Thus, there is a need to identify alternative natural drugs to overcome the several limitations of pharmaceuticals for treating breast cancer and prostate cancer. Some of the limitations include the disruption of the patients' immune system due to the severe side effects of synthetic chemical drugs. Moreover, cancerous cell metastasis is considered to be the main cause of mortality and morbidity. Recently, medical research has reported that the number of drug-resistant pathogens is increasing dramatically, although many new antibiotics are being produced (Batista, Duarte, Nascimento, & Simones, 1994). For example, many species of *Staphylococcus* (i.e., *Salmonella enteritidis*, *Salmonella typhi*, *Proteus*

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spp., and *Candida* spp.) are considered to be multidrug resistant (Appiah & Vlas, 2002; Barbour et al., 2004). The World Health Organization (WHO) has stated that 65% of people around the world prefer to use traditional herbal medicines (Gupta & Tandon, 2004). Historically, in India and the United States, the use of complementary alternative medicine has increased dramatically (Pandey & Madhuri, 2006). However, only a few plants have been investigated, and they might not have potential anticancer properties (Cragg & Newman, 2005). The ancient Egyptians also recommended the use of plants and plant extracts, such as castor oil and Palma Christus, as the most important way to treat diseases (Atta & Mounier, 2004; Scarpa & Guerci, 1982; Stubiger, Pittenauer, & Allmaier, 2003). Therefore, it is important to identify alternative natural drugs from plant origins that have less harmful side effects and are less costly than synthetic drugs.

However, few research studies have been conducted on the use of herbal medicines in the treatment of many types of cancers (Gutheil, Reed, Ray, & Dhar, 2012). To date, only a few herbal medicines have been analyzed chemically, even though their specific bioactive chemicals may have a potential anticancer effect. Many plants that belong to the Amaranthaceae family have several ethnobotanical effects, including *Achyranthes aspera* L., *Alternanthera philoxeroides* (Mart.) and Griseb., and *Gomphrena globosa* L. (Rahman & Gulshana, 2014). *Aerva javanica*, *A. javanica* (Burm.f.) Juss. ex Schult. (Amaranthaceae), is an herbal plant that grows to a height of about 30 cm to 1 m; it is commonly found in Africa and Asia and many countries around the world (Judd, Campbell, Kellogg, Stevens, & Donoghue, 2008). Traditionally, the plant has many applications; for example, it is used as a diuretic and in a diabetic demulcent. The extract of this plant is usually used to treat swelling and ulcers in domestic animals (Mufti et al., 2012). The plant's flowers and seeds are superficially applied as a paste to cure skin diseases, mitigate headaches, and to treat rheumatism. This led us to expect that some protective behavioral agents are present in the extract of *A. javanica* plants. In this study, *A. javanica* methanol extract (AJME) was investigated for its potency against breast cancer and prostate cancer. This article also discusses the antibacterial inhibitory activities of organic solvents against human pathogenic microbes. Methanol extract bioactive chemicals were evaluated using gas chromatography-mass spectroscopy (GC-MS).

Methods

Preparation of AJME

A. javanica leaves were collected from the abandoned area in the city of Abha in the Aseer Province of the Kingdom of Saudi Arabia in September 2018. The plant was assigned a voucher number and stored in the Biology Herbarium, Faculty of Science, Kingdom of Saudi Arabia. The leaves were air-dried for 1 week before being ground into a powder using a mortar and pestle. About 9 g of the powdered sample was placed in a Schott bottle containing 100 ml of methanol. The sample was incubated at 24°C for 3 days with agitation using a shaker. The sample was then filtered using Whitman paper to remove any solid materials. The filtrate was dried using a freeze dryer/lyophilizer to obtain the final product: AJME (Najmuddin & Romli, 2016). Five different concentrations of AJME were investigated by dissolving the extract in deionized water (0.01 µg/ml, 0.1 µg/ml, 1 µg/ml, 10 µg/ml, and 100 µg/ml).

Cell Culture

Human breast carcinoma cells (MCF-7) and human prostate cancer cells (PC3) were obtained from the American Type Culture Collection. The cells were sustained in Roswell Park Memorial Institute media. Roswell Park Memorial Institute media contains 100 units/ml of penicillin, 100 µg/ml of streptomycin, and heat-inactivated fetal bovine serum (10%) in a humidifier. The cells were kept in 5% (v/v) of CO_2 atmosphere at 37°C temperature. Twice a week, the cells were subcultured (Alarif et al., 2013).

Cytotoxicity and Viability of the Assays

Cytotoxicity

The cytotoxicity effect using five various concentration of AJME was examined for both the PC3 cell line and the MCF-7 cell line using sulforhodamine B (SRB) dye. The cells were collected using a 0.25% solution of Trypsin-EDTA and placed in 96-well plates at 2,000 cells/well. The cells were subjected to AJME for 72 hr, and then TCA (10%) was used as a fixative for 60 min at 4°C. The cells were exposed in a dark room to 0.4% of the SRB solution for 10 min after washing. Then, all the cells were washed with glacial acetic acid (1%). TRIS-HCl was used to liquefy the stained SRB cells after drying, and the intensity was measured at 540 nm. The dose–response curve of the compounds was analyzed using SigmaPlot version 12.0 software (Tolba et al., 2013).

Cell Viability

SRB staining was used to distinguish the viable cells from the dead cells. The viable or dead cells were ascertained with the density adjusted at 450 nm, using a microplate reader (Anthos Zenyth-200RT, Cambridge, UK), as described by Tolba et al. (2013).

Extraction of the Solvents

Fresh leaves of *A. javanica* were collected and either ground for the fresh extractions or completely air-dried at 21°C for the dried extractions. A mortar and pestle was used to grind the dry matter into a fine powder, then 25 ml of methanol, chloroform, petroleum ether, acetone, or hot water was added to 9 g of the ground materials. For the fresh extraction, the solvents were added to 19 g of the ground fresh *A. javanica* leaves. The flasks were kept in a rotary shaker instrument, at 370 rpm at 21°C for 48 hr. The extracts were filtered using Whitman paper, then dried in an oven at 63°C for 72 hr (Moustafa & Alrumman, 2015). The residue fraction of all the solvents were weighted, and then liquefied in 3 ml of sterile dimethyl sulfoxide. They were then placed in a rotary shaker at 190 rpm at 19°C for 72 hr and kept at 4°C (Salvat, Antonacci, Fortunato, Suárez, & Godoy, 2004).

Pathogenic Strains

Human pathogenic bacterial strains, including Gram-positive bacteria (*Micrococcus luteus* and *Staphylococcus aureus*) and Gram-negative bacteria (*Pseudomonas aeruginosa* and *Shigella flexneri*), were selected as the microbes. All the bacterial strains were first subcultured in nutrient broth and then incubated at 31°C for 24 hr.

Antibacterial Inhibition Assay

The antibacterial potency of the methanol, petroleum ether, diethyl ether, chloroform, acetone, and hot water extracts was determined using the agar well-diffusion method (Sulayli, Moustafa, & Eid, 2019). The prepared extracts were examined for their antibacterial activity against *S. aureus, M. luteus*, *P. aeruginosa*, and *S. flexneri*. Toward that end, 20 ml of sterilized nutrient agar was poured into disposable sterile Petri dishes, then kept at 24°C. The plates were inoculated with subcultured bacterial strains using a sterilized loop. A well with a 6-mm diameter was made using a sterile cork-borer, and then the well was filled with 100 µg/L of the plant extracts. The inoculated plates were then left at room temperature for 60 min to allow the plant extracts to diffuse completely. Dimethyl sulfoxide was applied as the negative control and Baneocin was applied as the positive control. Each experiment was repeated in triplicate, and the clear zones that formed on the agar media were measured in centimeters.

Analysis of AJME Using GC-MS

GC-MS was used to analyze the methanol extracts obtained from the leaves of *A. javanica* plants. The Clarus 500 Perkin–Elmer Gas Chromatograph with the Elite - 1 mass detector spectrometer was used; dimethyl poly siloxane (100%) was used to detect the presence of the extract, as indicated by the TR-V1 column (30 m × 1.8 µm × 0.32 mm) (Ezhilan & Neelamegam, 2012). Helium was used as a carrier, with a flow rate of 6 mm/min (2 µl injection volume and a split ratio of 5:1). The oven temperature was 35°C for 4 min, 110°C for 1 min, and then 45°C for 1 min, followed by an increase in the temperature to 170°C. To compare the unknown compounds with known compounds stored in the National Institute of Standards and Technology library, the phytocomponents were characterized and identified.

Statistical Analysis

The results were analyzed using two-way analysis of variance and Sidak's multiple comparisons test; the findings were reported as standard error (SE) ± of three replicates, and statistical significance was set as $p \leq .05$.

Results

Anticarcinogenicity of AJME

The results of the antiproliferative effect of AJME against prostate or breast cancer cell lines are shown in Figure 1(a) and (b), respectively. The extracts exhibited remarkable antiproliferative activity against the prostate and breast cell lines. The inhibition effect of the *A. javanica* extract was greater for the prostate cancer cells than the breast cancer cells at all the tested concentrations. The percentage of dead cells was 23.96% for the prostate cancer cells and 15.71% for the breast cancer cells at an extract concentration of 100 µg/ml. At an extract concentration of 0.01 µg/ml, the percentage of dead cells was 7.20% for the breast cancer cell line and 3.14% for the prostate cancer cell line. As the concentration of the extract increased (0.1 µg/ml, 1 µg/ml, 10 µg/ml, and 100 µg/ml), the percentage of dead cells increased from 7.163% to 84.289% than breast cancer. For the 0.01 µg/ml to 0.1 µg/ml extract concentrations, the percentage of viable cells in the breast cancer cell line was 15.6%. For concentrations ranging from 0.1 µg/ml to 1.0 ug/ml, the percentage of viable cells was 13.41%; for concentrations ranging from 1.0 µg/ml to 10 µg/ml, the percentage of viable cells was 15.89%, and for concentrations ranging from 10 µg/ml to 100 µg/ml, the percentage of viable cells was 32.19%. When the extract concentration was 0.01 µg/ml, the percentage of dead cells in the prostate cancer cell line was 4.03%. The percentage of viable cells increased as the concentration of the extract increased. The cell viability percentage curve was found to be different for prostate cancer than for breast cancer. When the extract concentration ranged

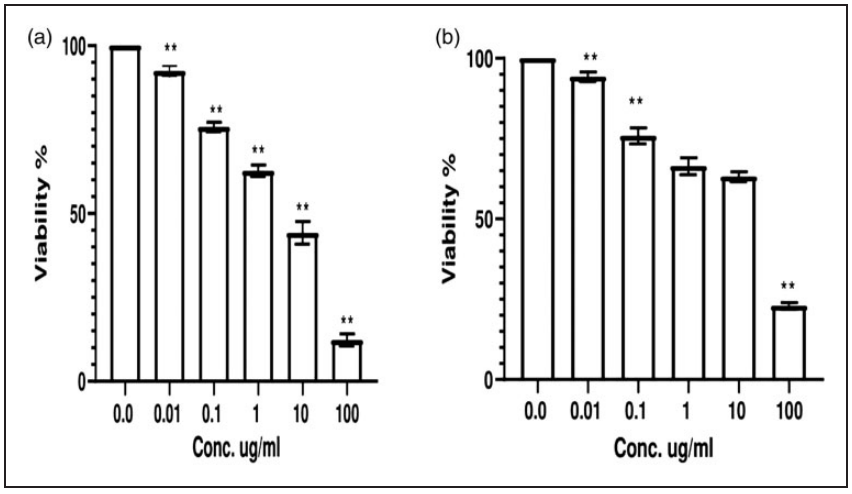

Figure 1. (a) Dose–response curves of *A. javanica* methanol extract against breast cancer. (b) against prostate cancer. Cells were treated with five different concentrations of *A. javanica* methanol extract for 72 hr. Error bars represent ± *SE*, $n = 3$; **a significant test result ($p \leq .05$).

from 0.01 μg/ml to 0.1 μg/ml, the percentage of viable cells for the prostate cancer cell line was 16.41%. When the extract concentration ranged from 0.1 μg/ml to 1.0 μg/ml, the percentage of viable cells was 10.61%. When the extract concentration ranged from 1.0 μg/ml to 10 μg/ml, the percentage of cell viability was 29.59%. When the extract concentration ranged from 10 μg/ml to 100 μg/ml, the percentage of viable cells was 42.88%.

Inhibitory Effect of Various Extracts of A. javanica

Figure 2 shows the inhibition activities of the *A. javanica* solvent extracts against the tested pathogenic bacterial strains. The results demonstrate that all solvents from the fresh and dried samples inhibited the activities of 100% of the tested microbial species. The antimicrobial activity of the solvent extracts was due to the structural variations among the precipitated bioactive phytocomponents in the *A. javanica* plants. The results demonstrate that the fresh solvent extracts were more potent against all the tested microbes than the dried extracts.

The analysis of variance results against *P. aeruginosa* showed that the difference in the inhibition zone between the dried and fresh extracts was 0.1205 ± 0.036 cm. The inhibition zone result was 1.670 cm for the dried extracts and 1.790 cm for the fresh extracts. The mean for the inhibition zone against *S. aureus* was 0.161 ± 0.016 cm; the inhibition result was 1.656 cm for the dried extract and 1.817 cm for the fresh extract. The mean for the inhibition zone of the extracts against *M. luteus* was 0.139 ± 0.036 cm; the inhibition zone result was 1.789 cm for the fresh solvent extract and 1.650 cm for the dried extracts. *S. flexneri* was affected by the dried extract (mean of the inhibition zone: 1.588 cm) while from fresh extract by 1.724 cm; the difference between these two extracts was 0.136 ± 0.0345 cm.

The *A. javanica* fresh and dried methanol and chloroform extracts showed excellent activity against all the tested microbes at 0.970 g/ml. For those extracts, the inhibition zone against *S. aureus* ranged from 2.457 ± 0.037 cm to 1.923 ± 0.059 cm for the fresh extract and from 2.357 ± 0.039 cm to 1.88 ± 0.003 cm for the dried extract. The petroleum ether and acetone fresh solvent extracts displayed moderate inhibition potency. For those extracts, the inhibition zone against *S. aureus* ranged from 1.78 ± 0.032 cm to 1.77 ± 0.055 cm. Against *S. flexneri*, the inhibition zone for those extracts ranged from 1.52 ± 0.0376 cm to 1.396 ± 0.021 cm. The inhibition zone against *S. aureus* for the dried extracts ranged from 1.36 ± 0.068 cm for the petroleum ether extract to 1.567 ± 0.051 cm for the acetone extract. The inhibition zone against *S. flexneri* ranged from 1.397 ± 0.032 cm for the petroleum ether dried extract to 1.197 ± 0.028 cm for the acetone dried extract.

Both the fresh and dried diethyl ether and hot water extracts demonstrated the lowest antimicrobial potency against the tested microbes. The inhibition zone for the diethyl ether dried extract was 1.417 ± 0.040 cm against *M. luteus* and *P. aeruginosa*, respectively. Against *S. aureus*, the inhibition zone for the diethyl ether dried extract was 1.13 ± 0.032 cm. The inhibition zone for the fresh diethyl ether extract ranged from 1.487 ± 0.018 cm against *M. luteus* to 1.21 ± 0.007 cm against *S. flexneri*.

The inhibition zone for the fresh extraction using hot water ranged from 1.23 ± 0.009 cm against *S. aureus* to 0.87 ± 0.038 cm against *S. flexneri*. The inhibition zone for the dried extraction ranged from 1.14 ± 0.023 cm

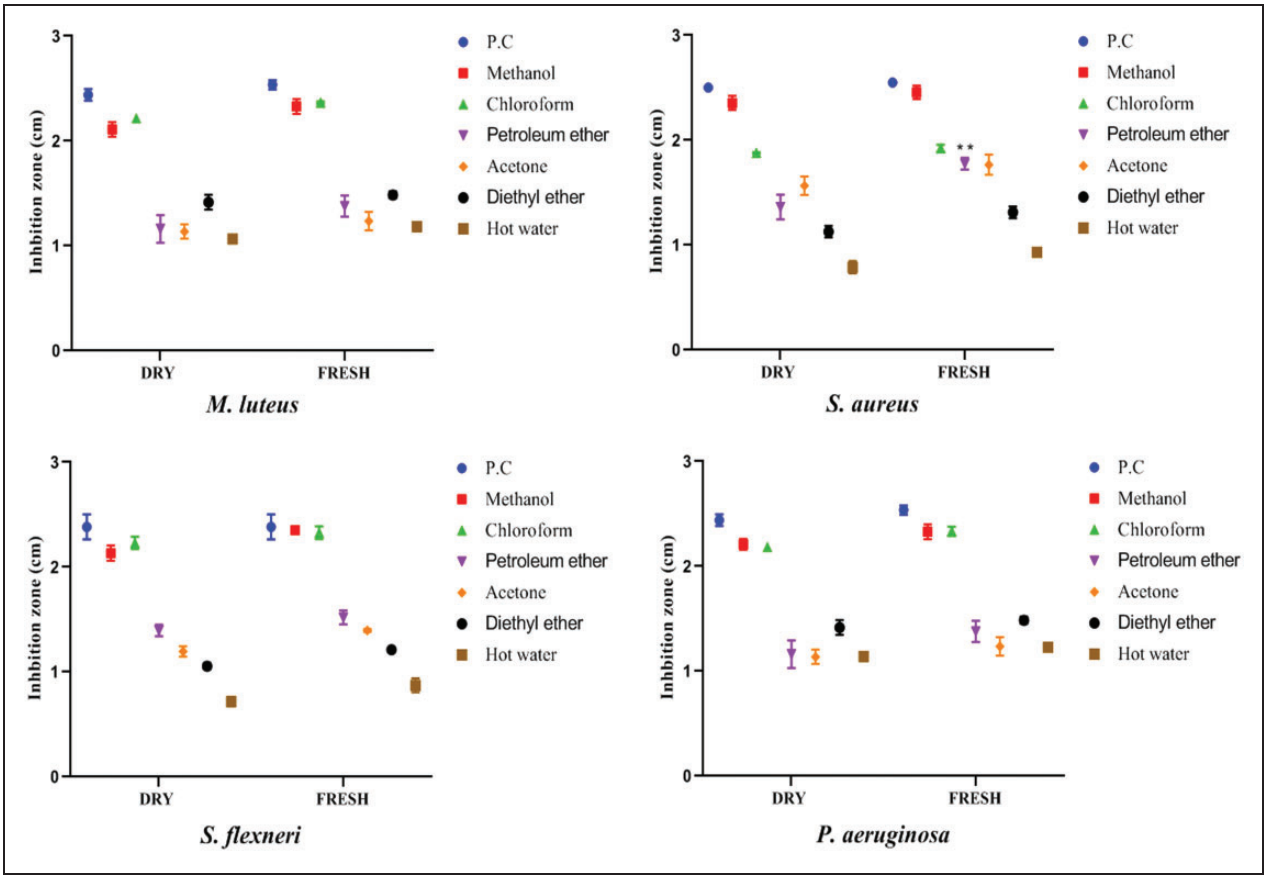

Figure 2. Antimicrobial activities of fresh and dry leaves of *A. javanica*. Error bars represent ± *SE*, $n = 3$; PC = Positive Control; **a significant test result ($p \leq .05$).

Table 1. Chemicals in Methanol Extract of *A. javanica*.

Compounds	Retention time (min)	Area (%)	Molecular weight	Chemical formula
Cyclohexasiloxane, dodecamethyl-	12.77	4.472	252.47	$C_{18}H_{36}$
2,6-Dimethyl-1-nitrobenzene	14.919	7.968	151.16	$C_8H_9NO_2$
Benzamide, 4-methoxy-N-((6-nitro-1H-benzimidazol-1-yl)methyl)-	15.708	1.364	326.312	$C_{16}H_{14}N_4O_4$
Diethyl phthalate	16.657	86.197	222.23	$C_{12}H_{14}O_4$

against *S. aureus* to 0.717 ± 0.026 cm to against *S. flexneri*.

Phytocomponents of AJME

Characterization and identification the active phytocomponents found in the AJME are illustrated in Table 1. Also, the retention time of the chemicals, as well as their molecular weight, molecular formula, and the percentage of their peak area, are shown. Four peaks were recorded from the GC-MS chromatogram. It was found that diethyl phthalate (86.197) is the most dominant phytocomponent in the AJME. The second most prevalent chemical found in AJME was 2,6-Dimethyl-1-nitrobenzene (7.958%). This extract was also found to have a small amount of cyclohexasiloxane, dodecamethyl- (4.472%), and benzamide, 4-methoxy-N-((6-nitro-1H-benzimidazol-1-yl)methyl)- (1.364%).

Discussion

Currently, the research target is to treat cancers using natural products obtained from plants because plants have many medicinal properties. In this study, the

cytotoxic impact of AJME on cancer cells was evaluated by applying five different concentrations of the extract to breast cancer cell lines and prostate cancer cell lines. The results show that all the studied concentrations had a significant impact on both types of cancer cells. The half maximal inhibitory concentration IC50 values vary among these two types of cancers, thus revealing variations in the effectiveness of the secondary metabolites. Breast cancer and prostate cancer are the two most dominant types of cancers in women and men, respectively. These two types of cancer vary anatomically in their physiological function, and the severe tumors are hormone dependent (Risbridger, Davis, Birrell, & Tilley, 2010). Hence, AJME could block the activity of the implicated hormones, to varying degrees, depending on the concentration of the extract.

Plant extracts have been used to cure cancer or suppress tumor growth; they have also been used as natural chemoprevention agents. For example, the anticarcinogenic characteristics of plants have been reported to treat fibrosarcoma in mice, Yoshida sarcoma, and ascites tumor cells (Reddy & Sirsi, 1969). *Albizzia lebbeck* plant extract was found to have a potent anticancer effect on sarcoma symptoms in mice (Dhar, Dhar, Dhawan, Mehrotra, & Ray, 1968). *Asparagus racemosa* has been used to treat epidermoid carcinoma in humans and *Boswellia serrata* has been used to treat nasopharynx epidermal carcinoma. *Erthyrina suberosa* has been used to treat sarcoma, *Anacardium occidentale* has been used to treat hepatoma, and *Euphorbia hirta* has been used to treat Freund virus leukemia. *Nigella sativa* has been used to treat Lewis lung carcinoma diseases, and *Peaderia foetida* has been used to treat epidermoid carcinoma diseases of the nasopharynx in humans. *Withania somnifera* has been used to treat various tumors (Dhar et al., 1968).

Recent studies have also shown promising results that are in agreement with our results. For example, 26 methanolic extracts from 18 plants grown in Kingdom of Saudi Arabia and Jordan were studied (Abdallah et al., 2018). That study found that some novel extracts, from *Phlomis viscosa* Poir and *Thymbra capitata* (L.), had antiangiogenic activities (Abdallah et al., 2018).

In this study, the chemical components in AJME were verified using GC-MS analysis to determine the possible mechanism of action. Previous studies have also analyzed plants to identify their chemical components to determine their biological activities. For example, the extract of fungus with cyclohexane and dodecamethyl was analyzed by GC-MS, and they were found to have anticancer properties against carcinoma cell lines (Abdel-Hady, Abdel-Wareth, El-Wakil, & Helmy, 2016). The compound could be used as a natural agent for hepatocellular carcinoma (Abdel-Hady et al., 2016). The derivatives of synthesized pyridine were examined for their analgesic, anti-inflammatory, anticonvulsant, and cytotoxic activity against a range of cell lines (Abd El-Galil & Mohamed, 2016). It was found that the derivatives had potent activities against prostate and renal cancer cell lines (Abd El-Galil & Mohamed, 2016). Nitrobenzene derivatives showed antibacterial activities against Gram-positive and Gram-negative bacterial strains (Nur Illane, Karimah, Adibatul, & Bohari, 2012). The chemicals isolated from *Sargassum wightii*, a type of seaweed, were tested against the cancer-specific HER2 and HER2/neu (Balachandran, Ajay Kumar, & Parthasarathy, 2016). It was found that diethyl phthalate had efficient activities against the HER2 receptor of breast cancer (Balachandran et al., 2016).

Herbal plants have also been investigated in detailed pharmacological studies to obtain new pharmaceutical compounds and to develop new drugs (Matu & van Staden, 2003). These medicinal plants may prove to be new and effective agents against pathogenic bacterial and viral activity (Coelho de Souza, Haas, Von Poser, Schapoval, & Elisabetsky, 2004). An estimated 4 billion people around the globe use traditional herbal medicines as a part of their primary healthcare regime (WHO, 2003). It has been reported that there is an increase in the emergence of multidrug-resistant bacteria due to intensive use of synthetic drugs (Katsumi, Naoe, Matsushitam, Kaibuchi, & Schwartz, 2005). In many parts of the world, the potency of plant extracts as antibacterial agents has been studied (Duru & Onyedineke, 2010; Nasar-Abbas & Halkman, 2004; Nwachukwu, Allison, Chinakwe, & Nwadiaro, 2008). However, only about 20% of the plants that have been recorded have been subjected to pharmacological or biological investigation (Mothana & Lindequist, 2005).

This study's results found that solvent extracts from the leaves of *A. javanica* had antibacterial activities against *M. luteus*, *S. aureus*, *P. aeruginosa*, and *S. flexneri*. The methanol and chloroform extracts were found to have the most potent antibacterial activity against all the tested microbes. Our findings are in line with the results reported by Chatha, Anwar, Manzoor, and Bajwa, (2006) and Siddhuraju and Becker (2003). Those studies reported that the highest phytocomponents extracted from rice bran and *Moringa oleifera* leaves were obtained with a methanol extract. The methanolic extract of *Alchornea cordifolia* was found to have more potent antibacterial activity than other extracts tested against *Escherichia coli* and *P. aeruginosa* (Okoye, Uba, Uhobo, Oli, & Ikegbunam, 2014). Sati and Joshi (2011) reported that the methanol extract of *Ginkgo biloba* had the highest antibacterial activity against microbial strains in comparison to other extracts. The chloroform extract of *N. sativa* was found to have potent antibacterial activity against multidrug-resistant human microbes (Alam, Yasmin,

Nessa, & Ahsan, 2010). However, chloroform extracts from *Antrodia camphorata* were reported to have the lowest minimum inhibitory concentration (MICs) against dental bacteria (Lien et al., 2014).

In this study, the acetone and petroleum ether extracts showed moderate antibacterial activity against the tested microbes; this finding is in agreement with the data reported by Tedela, Adebiyi, and Aremu (2014). It was found that the *Nicotiana tabacum* and *Bryum coronatum* acetone extracts had moderate effects against *S. aureus* and *Klebsiella pneumonia*. In this study, the hot water extracts were found be the least effective in inhibiting the activity of the tested microbes. A previous study showed that aqueous extraction is not as potent as extractions using organic solvents. Andriana et al. (2019) reported that hot water extract of *Tridax procumbens* L. showed no inhibition activity against the tested microbes. This study confirmed earlier observations that either polar or nonpolar solvents had the capacity to produce a large number of bioactive molecules (Sujatha & Suresh, 2013; Tiwari, Kumar, Kaur, Kaur, & Kaur, 2011). Thus, the solvents used in this study play a key role in precipitating biologically active phytocomponents.

Differences in the susceptibility of bacterial strains to the tested extracts may be due to structure of the cell walls. *A. javanica* solvent extract had a more pronounced effect on growth of Gram-positive bacteria, especially *S. aureus*, than the other tested bacteria. Many studies have reported on the susceptibility of Gram-negative bacteria. Moustafa, Hesham, Quraishi, and Alrumman (2016) and Alamri and Moustafa (2010) found the same trends for the behavioral activities of extracts against a range of human pathogenic microbes. Gram-negative bacteria have an outer membrane and a unique periplasmic space that is not present in Gram-positive bacteria; the outer membrane of Gram-negative bacteria is a hydrophilic surface forming a barrier to the penetration of numerous antibiotic (Gao, Belkum, & Stiles, 1999; Nikaido, 1994). However, not all solvent extractions completely follow the trend described earlier. It is not known exactly why, in some cases, Gram-negative bacteria are more susceptible than Gram-positive bacteria. This could be related to the presence of some specific anti-Gram-negative substances in the extracts, as previously described (Shan, Cai, Brooks, & Corke, 2007).

To some extent, antimicrobial treatment of these kinds of bacteria can reduce morbidity, mortality, and disease transmission. However, a high prevalence of clinical pathogens, such as chloramphenicol, streptomycin, ampicillin, and sulfonamides, are resistant to antibiotics (Kozak et al., 2009). This study's findings uphold the importance of using *A. javanica* extracts instead of synthetic drugs, thereby supporting the use of traditional, medicinal plants.

It is believed that, to some extent, antimicrobial activities are caused by the phytoconstituents present in the plant species, such as turmeric oil (Apisariyakul, Vanittanakom, & Buddhasukh, 1995), diterpenes (Batista et al., 1994), and phenolic acids (Fernández, García, & Sáenz, 1996). The production of chemicals from plants has also been reported to be linked to many factors, including natural genetic variation in the plant, prevailing climatic conditions, and the physiological traits of the plant (Moustafa et al., 2016).

Generally, the antibacterial potency of the fresh *A. javanica* extracts was more effective than the dried extracts in terms of the zone of inhibition. This could be because the active chemicals in the plant and their volatile oils might have been subjected to degradation during the drying process. Previous researchers have found that antibacterial activity was clearly decreased after drying, for example, in *Zingiber officinale* and *Lippia gracilis* plants (Bitu et al., 2012; Sasidharan & Menon, 2012). In conclusion, the methanolic extract of *A. javanica* showed promising properties against breast cancer and prostate cancer, and it could be a natural anticancer agent. These findings ensure that *A. javanica* extracts could be an important source of bioactive components with broad-spectrum antimicrobial properties.

Implications for Conservation

Additional scientific research is needed to obtain pharmaceutical knowledge about weeds and plants growing in Saudi Arabia and many other countries. This article provided important information about the pharmaceutical applications of *A. javanica* extracts, obtained from either organic solvent extractions or hot water aqueous extraction. In the future, *A. javanica* is a plant that can, potentially, be used as an important and safe medicine to treat cancers; it could also be used as a natural antibiotic agent. This finding supports the folklore knowledge that plants can be used to treat a variety of ailments, including headaches, rheumatism, and kidney stones. The study's results also showed the presence of important phytocomponents in the *A. javanica* extracts that provide biological benefits; however, additional research is needed to verify that finding. The isolation and characterization of phytochemicals will help determine if they work synergistically or individually. Therefore, we believe that this study contributes to the documentation of scientific knowledge about the medicinal properties of plants. In addition to the global dramatic fluctuations in climate change, many countries are enacting a series of socioeconomic changes as part of their development programs. Consequently, large areas of virgin land are being turned into urban and agricultural areas, and the number of endangered plant species is increasing. Therefore, we suggest that the central government

should develop and implement a plan to preserve those plants, save them from extinction, and increase the public's awareness about the need for environmental protection.

Declaration of Conflicting Interests

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