QATAR UNIVERSITY

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ANTIMICROBIAL AND CYTOTOXIC ACTIVITIES OF SELECTED QATARI FLORA

BY

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ABSTRACT

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Title: Antimicrobial and Cytotoxic Activities of Selected Qatari Flora

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Conventional medicine has been challenged by various issues, including drug resistance and safety. On the other hand, Complementary Alternative Medicine has been increasingly gaining the interest of the scientific community and the public for its efficacy and safety. Therefore, the search for cytotoxic and antimicrobial agents from plants has been booming in the last few decades. This study was designed to investigate the anticancer and antifungal activities of the crude extracts (aqueous and organic) from four native Qatari plant species; *Aerva javanica*, *Limonium axillare*, *Salsola soda* and *Suaeda vermiculata*. The antifungal activity of their crude extracts on the species; *Alternaria alternata, Cladosporium sphaerospermum, Fusarium oxysporum,* and *Botrytis cinerea*, was assessed *in vitro* by two methods; poisoned food and agar diffusion method. While aqueous extracts were not effective, the ethanolic extracts of all plant (aerial parts) were active against all of the tested fungi, and the concentration of 10 mg/ml was enough to inhibit fungal growth. Extracts of *L. axillare* had the highest activity, with minimal concentration values between 5 and 2.5 mg/ml. The anti-cancer activity of the crude extracts was also assessed on the MCF-7 breast cancer cell line, indicating that with water crude extracts of *S. vermiculata* at concentrations of 400 and 300 µg/µl were the only effective doses worked against MCF-7 cell proliferation. A comprehensive study is needed to assess the anticancer activity of the *S. vermiculata* crude extract, using normal human epithelial breast cells and various additional breast cancer cell lines. Last but not least, fractionation will be carried out to identify the bioactive ingredients that are responsible for both anti-cancer and anti-fungal activities.

DEDICATION

For my husband, Mohammad, my son, Faris

And For my parents, Ahmad and Wafa'..

I dedicate this work ..

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1. INTRODUCTION

Despite the great development of medicine, plants are still considered the main contributor to health care. Plants contain a wide range of chemicals and bioactive compounds that used for the development of pharmaceutical drugs. In addition, their extracts have also been used in traditional therapies for thousands of years (Hayta et al., 2014). As there is an increase emergence of infection causing microbes (in humans, plants and animals) and toxins, it is important to investigate the possibilities that can treat these harmful pathogens with lesser sides effects. One of these possibilities is the use of medicinal plants.

Breast cancer (BC) is the most common cancer among women worldwide. It has an incidence of 31% in Qatari women aged below 60 years (Qatar Cancer Registry, 2008). Cancer is a result of gene mutations that lead to genetic unstability, abnormal cell growth, and tumorigenesis. (Urry, 2016). Although inherited mutation in BC genes might cause 5-10% of breast tumors, the majority of breast tumors (90-95%) are caused by environmental agents, including chemical carcinogens, viruses, and radiation (Urry, 2016). The current treatment modalities of BC include chemotherapy, radiotherapy, and surgery. However, there are many unwanted side effects such as hair loss, drug resistance, suppression of bone marrow, neurologic dysfunction, cardiac toxicity and gastrointestinal lesions (Hosseini & Ghorbani, 2015). Thus, despite extensive research in conventional medicine (CM) and the fast progress of technology, the effectiveness of current treatment modalities against cancer is being challenged by various issues, including drug resistance and drug safety. However,

Complementary and Alternative Medicine (CAM) has been gaining an increasing interest in the scientific community as well as the public for being natural, safe and effective.

On the other hand, fungi, which are eukaryotic microorganisms, play an important role in decomposition of organic material and recycling it into the environment in usable forms for other organisms, such as plants and animals. However, they can attack plants, spoil food, and afflict humans with diseases such as athlete's foot and other maladies (Urry, 2016). The main treatment of these microorganisms in agriculture includes chemical fungicides. However, these chemicals pose challenging problems, including development of new fungal resistance that could be very toxic to a human being, and living organisms (Salhi et al., 2017).

Therefore, the search for effective and safe anticancer and antifungal agents is essential. In fact, Complementary Alternative Medicine (CAM) research has been booming in the last decade, aiming to identify bioactive compounds as natural source for therapeutic drugs, and further characterize their mode of action(Hosseini & Ghorbani, 2015). CAM involves a wide range of therapeutic approaches, one of which is herbal medicines. It has evolved over thousands of years, and it is widely practiced around the world (Mainardi, Kapoor, & Bielory, 2009). One of the most interesting areas has been the identification and isolation of the bioactive constituents from dietary herbs and then determine their anticancer and antimicrobial properties. For example, in cancer treatment, a CAM type called "chemo-prevention therapies" deal with phytochemicals that are derived from dietary herbs, and are used to treat and prevent cancer (Mainardi et al., 2009). Curcumin (diferuloylmethane), the major constituent of the root of *Curcuma longa* L. plant, has been established as an anticancer agent against colon, breast, lung and brain tumors (Wang et

al., 2012). Cyanidin, which is a bioactive compound in red berries, such as grapes, apples, and red onion inhibits cell proliferation of colon cancer cells (Wang et al., 2012). In addition, a combination of six phytochemicals has been found to be effective as against breast cancer cell growth (Ouhtit et al., 2013). Furthermore, a number of studies showed that various plant extracts had antimicrobial activities has shown that many plants have active compounds that can inhibit the growth of different microbial species, such as *Escherichia coli*, *Pseudomonas auruginosa*, and *Staphylococcus aureus* (Barbieri et al., 2017). For example, the organic and aqueous extracts from the leaves of *Annona squamosa* have been reported as an antifungal agent against *Alternaria alternata, Aspergillus niger, Fusarium solani, Candida albicans* and *Microsporum canis* species (Kalidindi, 2015). The Garlic oil was reported as antifungal agent against *Penicillium funiculosum* (Li, Shi, Liang, Huang, & Chen, 2014). Also, the essential oil that isolated from *Origanum floribundum* shown a strong antifungal activity against *Candida albicans* (Gormez & Diler, 2014).

The observations described above prompted us to focus on four local Qatari medicinal plants, and test the effect of their crude extracts on breast cancer (BC) cell proliferation and on a range of fungal species. Our main goal is to determine the biological

activities (antifungal and cytotoxicity) of crude extracts from four native Qatari plant species; *Suaeda vermiculata, Limonium Axilare, Salsola soda,* and *Aerva javanica*.

2. LITERATURE REVIEW

2.1 Medicinal Plants

According to WHO (2008), in developing countries, 80% of the population depend on traditional herbal medicine as an alternative for primary health care. Until the middle of 19th century, herbs were the core of the medicine used by the humans, and even nowadays, their roles in medical science are still relevant (Hayta, Polat, & Selvi, 2014; Krishnaiah, Sarbatly, & Nithyanandam, 2011). Globally, about 60,000 plant species are known for use in traditional and modern treatment systems (Hayta et al., 2014). In herbal medicine system, particular plants with complex formulations were used to treat diseases (Akram et al., 2014).

The active constituents of plant extracts, have been extensively reviewed, especially in antioxidant and antimicrobial activities (Al-Jaber, Awaad, & Moses, 2011). The phyto-therapeutics of these extracts having low molecular mass compounds have been successively used as a reactive oxygen species, since ancient times. Many plant species can play an important role as an antioxidant agent by acting as lipid peroxidation inhibitors and radical scavengers (Al-Jaber et al., 2011). The results of the majority of the experiments of the antioxidant and antimicrobial assays as well showed that these activities are because of many secondary metabolites, particularly phenolic compounds, such as flavonoids and tannins (Al-Jaber et al., 2011). To date, more than 1000 different extracts of

phytochemicals from many daily food plant species have been successively evaluated against several human pathogens, such as bacteria, fungi, and viruses (Barbieri et al., 2017).

In the middle east, particularly in Arabic Peninsula, the medicinal plant distribution and status is still lacking with research works. However, Saudi Arabia has the richest biodiversity in the medicinal plants, which occupy more than 50% of the total species existing in all area (Kuete et al., 2013; Baydoun et al., 2015).

2.2 Bioactive and Phytochemical Constituents of Medicinal Plants

Phytochemicals are big groups of natural chemical compounds found in plants that give the plants their flavor, aroma, color, and texture. They are distributed mainly in fruits, vegetables, whole grains, legumes, seeds, nuts and in plant-based beverages, such as tea (Barbieri et al., 2017). The developmental history of these compounds began over thousands of years to defend organisms from the effects of viruses, fungi, bacteria and free radicals (Mufti et al., 2012). Due to their important roles in innovation new compounds such as herbal remedies are designed for treatment and diagnosis of many diseases (Barbieri et al., 2017; Mufti et al., 2012). Based on their chemical structure; phytochemicals can be classified into four major groups: alkaloids, polyphenols, terpenoids and sulfur-containing phytochemicals (Barbieri et al., 2017).

2.2.1 Alkaloids

Alkaloids are nitrogenous bases in organic compounds varying from one chemical structure to other. Alkaloids have been used in medicine for very long time (Barbieri et al.,

2017). Many types of alkaloid structures are reported to be a strong inhibitor of superoxide radical (O^{-}_{2}) scavenger, singlet oxygen species ($^{1}O_{2}$) and lipid peroxidation. Tetrandrine alkaloid, which is isolated from *Stephania tetrandra*, played a strong role in scavenging of O^{-}_{2} and OH^{-}, and in inhibition of 5-lipogenase (Al-Jaber et al., 2011). In addition, sanguinarine; an alkaloidal derivative from rhizomes of *Sanguinaria canadensis*, is reported having antimicrobial and anti-inflammatory activities (Barbieri et al., 2017). The roemerine alkaloid type that isolated from *Papaver rhoeas* plant species was reported to have strong antimicrobial activity against *Staphylococcus aureus* and *Candida albicans* (Coban et al., 2017). Also, total alkaloids extract from *Annona hypoglauca* have shown a strong anticancer activity against colon and breast cancer, and antimicrobial activity against *Staphylococcus aureus* and *Enterococcus faecalis* (Rinaldi et al., 2017).

2.2.2 Diterpenes

The phenolic diterpenes, such as rosmaridiphenol, carnosic acid, resmanol, epirosmanol and carnosol, isolated from *Rasmarinus officinalis*, were shown to be an important protective player against oxidative stresses in the biological systems (Al-Jaber et al., 2011). Other antioxidants obtained from *Prodocarpus nagi*, such as totarol, sugiol and totaradiol 19-hydroxytotarol totaral, were also evaluated, which the inhibits linoleic acid auto oxidation (Haraguchi et al., 1997).

2.2.3 Polyphenols

A vast number of edible plants contain polyphenol compounds, especially in fruit, vegetables, nuts, seeds and beverage such as tea and coffee. Polyphenols, both; flavonoids and non-flavonoids, have been evaluated therapeutically as a chemo-preventive agent due to their strong direct antimicrobial actions (Barbieri et al., 2017). In selected plant species, the relationship between antioxidant activities and total phenolic content was directly linked (Valdez et al., 2000). Many types of polyphenols, such as flavonoids, hydrolysable tannins, and phenolic acid, were shown to have antimutagenic and anticarcinogenic effects (Bravo, 1998). Also, their activities were shown in scavenging capacity of free radicals and chelating of metal (Al-Jaber et al., 2011). In addition, some polyphenols have been reported as a mutagenic agent in microbial assays and promoters in inducing carcinogenesis of skin in the presence of other carcinogens (Al-Jaber et al., 2011).

2.2.4 Flavonoids

Flavonoids have a big group of compounds, that can be found in legumes, vegetables, and fruits (Barbieri et al., 2017). Flavonoid acts as free radical scavengers in the body that neutralize all types of oxidizing radicals by chelation, the most important function of flavonoids. The chelating activities of flavonoids in our bodies prevent the metal ions from being available for oxidation by chelating or binding to them (Al-Jaber et al., 2011; Barbieri et al., 2017). Also, it has phenolic groups that can break off the antioxidant due to its' electron-donating capacity (Merfort et al., 1996). Flavonoids are known to have anti-inflammatory, antiviral, antibacterial, antiallergic and anticancer

activities (Barbieri et al., 2017; Lin et al., 2008). Pharmacologically, flavonoids have protecting effects on carcinogenesis by antioxidation of microsomal mono-oxygenase, that have a detoxifying action, in which it can inhibit the neoplastic effects of chemical carcinogenesis (Al-Jaber et al., 2011). Also, they have chemo-preventive activities against many diseases associated with oxidative stress, such as neurodegenerative and cardiovascular diseases (Lin et al., 2008). For example, *Astragalus* spp. reported to have flavonoids that attributed to their biological activities (Gorai et al., 2016). Flavonoid and its derivatives, especially isoflavonoids, that isolated from *A. lasioglottis* have been shown to be antimicrobial, estrogenic and insecticidal activities (Gorai et al., 2016). In addition, flavonoid extraction from almond skin exhibited antimicrobial activity against Gram-positive bacteria and *Salmonella enterica* (Mandalari et al., 2010). Also, flavonoid content in *Jovibarba heuffelii* was attributed antimicrobial activity against *Penicillium chrysogenum, Aspergillus restrictus* and *Acremonium chrysogenum* fungus species (Dimitrijevic et al., 2010).

2.2.5 Anthocyanins and Anthocyanidins

Anthocyanins were recorded as a strong medicinal compound, in comparison with other plant compounds. Anthocyanins compounds are responsible for the colors of many fruits, vegetables, berries and flowers (Barbieri et al., 2017). Therapeutically, anthocyanins are used as a powerful antioxidant in eye and heart linked health complications (Al-Jaber et al., 2011). The richest sources of anthocyanins are berry nectars, such as elderberries (*Sambucus cerulean*), blueberries (*Vaccinium myrtillus*), cranberries (*Vaccinium*

macrocarpon) and prunes (*Prunus domestica*). In addition, some of the dietary food, such as apples (*Malva pumila*), eggplant and red cabbage, are common food items that contain anthocyanins (Al-Jaber et al., 2011).

Proanthocyanidins; a family type of anthocyanidin (so-called condensed tannins) have antioxidant activities, particularly against many cancer-causing agents and heart disease (Milner, 2001). Proanthocyanidins are found in many types of berries, such as blueberry, elderberry, and cranberry, grapes, apples and prunes (Al-Jaber et al., 2011).

2.2.6 Phenolic acids

Phenolic acids have been reported as active antioxidants. They are rich especially in sweet potatoes and many plant extracts, such as caffeic acid and chlorogic acid and its derivatives and isomers (Al-Jaber et al., 2011). For example, *Brosimum alicastrum* is reported to have high content of phenolic acid that act as free radical scavenger agent (Ozer, 2017). Also, *Acacia nilotica, Acacia catechu,* and *Albizia lebbeck* were reported to have rich content of phenolic acids, which contributed to their biological activities including anticancer and antioxidant activities (Sulaiman & Balachandran, 2012).

2.2.7 Tannins

Tannins are phenolic compounds that are secondary metabolites of plants. They have a big structural and concentration variation within plant species. In vascular plants, tannins are present in two forms; hydrolysable and condensed forms (Hernes et al., 2001). The condensed tannins are also called proanthocyanidins (PAs). PAs have shown a great

value of antioxidant capacity in nutrition and medicine, that is related to their scavenging capacity of radicals. These properties have been used in lipid oxidation-reduction, against heart diseases (Ricarda Da Silva et al., 1991). However, most of the hydrolysable tannins have a stronger antioxidant activity when compared with condensed tannins. Tannins activities in plants have been shown in (1) suppression of the oxidation of ascorbic acid by blocking of Ca^{2++} or by radical scavenging activities, such as geraniin, in (2) lipid peroxidation inhibition, such as geraniin, isoterchebin and pedunculagin, or in (3) scavenging of free radicals especially in which they have a significant inhibitory actions on galactosamine carbon tetrachloride hepatic cytotoxicity and, such as licorice phenolics (Al-Jaber et al., 2011).

2.3 Antimicrobial Activities of Medicinal Plants

The crude extract from the whole *Aerva lanata* plant, especially methanol and ethyl acetate extracts, showed antimicrobial activities against bacteria (*E. coli, Bacillus subtilis, B. cereus, Staphylococcus aureus* and many type of genus *Shigella*) and fungi (*Aspergillus fumigatus, A. niger, Hensinela californica* and *Candida albicns*) (Chawla, 2012). Also, the aqueous extract of *Aerva persica* has a wide range of antimicrobial activities, aqueous extract from leaf showed antibacterial activity against *Salmonella typhi* (Chawla, 2012). Additionally, the same study reported that the aqueous extracts of stem and leaf and alcoholic extracts of flowers showed antibacterial activity against *S. aureus* and aqueous and alcoholic extracts of the whole plant showed total inhibition of *Macrophomina phasolina* (plant pathogen fungal species) growth. *Aerva bovei* ethyl acetate extract showed

antimicrobial activities against *E. coli, Aspergillus niger* and *Saccharomyces* (Chawla, 2012).

The saponin fraction that obtained from Quinoa seeds showed inhibition activity on the growth of *C. albicans* at 50 ug/ml (Mroczek, 2015). Crude extract from *Suaeda maritima* is reported as an antibacterial agent (Cybulska et al., 2014). *Paeonia emodi* methanol and n-hexane fractions showed antifungal activities against *A.niger* and *A. flavus* (Mufti, 2012). Chloroform extract from aerial parts of *Salsola rosmarinus* shown significant effects against *Bacillus subtilis* (Cybulska et al., 2014; Wang et al., 2012). Ethanol extracts from *Smilax campestris* showed antifungal activities against *Cryptococcus gattii* and a vast range of *Candida* species, such as *C. albicans, C. parapsilosis, C. glabrata* and *C. tropicalis* (Morais, 2014).

Antifungal activities of essential oils of *Stachys pubescens*, *Thymus kotschyanus*, *Thymus daenensis,* and *Bupleurum falcatum* have shown strong activity against many fungal species, such as *Aspergillus flavus, Alternaria alternata* and *Fusarium oxysporum*. In addition, the antifungal activities these plant extracts showed almost the similar activity as of amphotericin B, a commercial fungicidal agent (Mohammadi et al., 2014). Miller et al. (2015) studied the essential oils of *Lippia graveolens* and *Origanum vulgare*. They reported noticeable antimicrobial activities against *Candida albicans, S. aureus,* and *E. coli*. Furthermore, these plants' oil can inhibit the growth of *C. albicans*.

Bitter apple (*Citrullus colocynthis*), which is a desert plant recorded in many Middle East countries including the Gulf, have shown antifungal and antibacterial activities in traditional uses (Elansary et al., 2018). In addition, *C. colocynthis* fruit coat and seeds, *Senna alexandrina* pods and *Asparagus aethiopicus* leaves showed general antifungal

activities, that can be referred to the high number of phenolic compounds in those plants. Furthermore, Elansary et al. (2018) reported that *C. colocynthis* has strong antifungal activity against *Aspergillus fumigatus* and *Mucor spp.* Moreover, the high robinin content in *Robinia pseudoacacia* has been reported to have high antifungal activities.

2.4 Cytotoxic Activities of Medicinal Plants

Various studies showed a cytotoxic activity of *Amaranthaceae* saponins against various cancer cell lines where the structural-functional relationship was explored (Mroczek, 2015). *Salicornia bigelovii* contains Bigleovi A saponin type, that is known inhibit HepG2 (liver) and MCF-7 (breast) (Mroczek, 2015) tumor cell proliferation; *Achyranthes* saponin has induced apoptosis of BC cells (Mroczek, 2015). *Aerva lanata* showed strong cytotoxic activities in methanolic and ethyl acetate fractions (Chawla, 2012). In addition, *Salsola* species were evaluated along with their phytochemicals. These species encompass different types of flavonoids, simple tertahydroisoquinoline (TIQs), coumarines, acetophenones, and sterols. Tetrahydroisoquinolines and isoquinoline alkaloids have cytotoxic effects against tumor cell lines (Tundis, 2009).

Flavonoids, which are extracted from *Halocnemum strobilaceum*, have shown strong antioxidant activities (Cybulska et al., 2014). Ethanolic extracts of *Saueda maritima* leaves showed free radical scavenger (antioxidant) activity (Cybulska, 2014). Curcumin, which is a diferuloylmethane phenol found in *Curcuma longa* roots revealed anticancer activity (Elansary et al., 2018).

Artemisia monosperma stem and leaves extracts showed significant anticancer activities against BC cell lines, especially by polyacetylene dehydrofalcarindiol bioactive

compound. However, *Artemisia* genus is used in folk medicine due to its biological activity, such as antibacterial, antiviral, antifungal, cytotoxic and antioxidant activities (Solowey, 2014).

Plants such as; *Senna alexandrina*, *Asparagus aethiopicus*, and *Citrullus colocynthis* were reported as antioxidant agents. Their extracts contain many different secondary metabolites, such as benzoic acid, phenylalanine, and robinin. In addition, *A. aethiopicus* leave extract contain many other phenolic compounds that have been reported as strong antioxidant agents, such as rutin (Elansary et al., 2018).

Smilax campestris ethanolic, and butanolic fractions of its areal parts exhibit significant antioxidant activity in scavenging of 1,1-diphenyl-2-picrylhydrazyl radical, in comparison with 2,6-di-tert-butyl-4-methylphenol, a commercial antioxidant (Morais et al., 2014).

Salsola imbricata was reported to have high hepatoprotective and antioxidant activities mainly because of the high content of many different phenolic compounds including aglycone, flavonoids and phenolic acids, particularly rosmarinic acid and quercetrin glycoside. Rosmarinic acid and quercetrin glycoside are previously reported as strong antioxidant agents (Shehab, Abu-Gharbieh, & Bayoumi, 2015).

Traditionally, *Brucea javanica* has shown a wide range of biological activities, such as anticancer, antitumor, antidiabetic, anti-inflammatory, antiviral and antimalarial activities. Moreover, a recent study demonstrated that *B. javanica* extract had shown a strong cytotoxic effect on colon cancer cells (Bagheri et al., 2017). Also, the same

researchers reported that this extract is nontoxic to normal human cells (Bagheri et al., 2018).

2.5 Qatari Flora

The state of Qatar is a small peninsula, about 11,430 km^2 in area between 24°40' and 26°10'N and 50°45' and 51°40'E, with 900 km of coastline, and it connected to the Arabian Peninsula from the south of Saudi Arabia in a wavy landscape with rocky and conglomerate hamadas (hazm, misfah), depressions (rodat), rocky ridges (such as Jabal Dukhan), runnels and wadies, subkhas and sand formations (Babikir, 1990; Kurschner, 2018). The natural topography of Qatar is mainly flat and desert. In the south, the topography is mostly rocky hills and sand dunes, and along the coast, saline swampy mud flats are common (Babikir, 1990). The coastline presents uneven outline with numerous reefs, island, inlets and large salty-sandy depression (*sabkhas*) areas (Kurschner, 2018). Qatar is hot, dry and surrounded by a shallow, hypersaline, semi-enclosed sea. The climate in Qatar is recognized as hot, dry summer with high temperature, high relative humidity and low precipitation (Babikir, 1990).

The diversity of Qatari flora was investigated as early as 1970 when Mrs. Cheryl Wilson visited Qatar and started developing botanical drawings that showed the floral diversity of Qatar. The major collections and research on the Qatari floral diversity were carried out in 1981 and 1983 (Abdel Bary, 2013). A huge gap has occurred since 1983 till now. During this period, loss of some natural habitats as well as species might have occurred. Similarly, during this period, alien and invasive species have also been introduced in Qatar. Hence, the impact of them should also be studied (Abdel Bary, 2013).

So far in Qatar, there are no species that are included in the world list of endangered species, but it has been noted that some species might be susceptible to complete disappearance from the flora if they were continuously exploited (Abdel Bary, 2012).

To the date, a total number of 371 species are found in Qatar that belongs to 236 genera. These species are from 62 families out of which 53 are dicotyledons, 8 are monocots, and 1 is a gymnosperm (Abdel Bary, 2013). Four genera of parasitic plant species are also known in Qatar belonging to three families; Cuscutaceae, Orobanchaceae, and Cynomoriaceae. In Qatar, there are three types of species recognized as non-flowering plants. Three species of non-flowering plants are recently recorded as bryophytes by Kurschner and his college (2018). In addition, ferns and gymnosperms are another non-flowering plants species existing in Qatar. However, 400 species of flowering plants are known to be present in Qatar out of which 270 are truly native to Qatar (Abdel Bary, 2013).

The distribution of native vegetation covers whole Qatar region as they are more spread around in three major areas; Northern, Central and a belt that starts from the central west to Southern of Qatar. The inland vegetation in Qatar is sparse where it contains herbaceous plants and dwarf shrubs (Abulfatih et al., 1999). Moreover, the most popular vegetation species that covers Qatari surface area from trees to shrubs and to perennial plants are *Acacia tortilis, Acacia ehrenbergiana, Ziziphus nummularia, Prosopis spp, Lycium shawii, Tamarix L., Leptadenia pyrotechnica, Cybopogon spp, Pennisetum spp, Cyperus L.* and *Panicum spp* (Al-safran, 2014). Qatar and neighboring gulf countries

including the United Arab Emirates, Eastern regions of Saudi Arabia, Bahrain, Kuwait, and Oman have good of similarities of flora and vegetation (Al-safran, 2014).

Distribution of vegetation in arid environment is controlled by several principles; positive correlation between vegetation and precipitation; positive correlation between plant size and rainfall; diversity of plant species are related to single humped curve richness; soil heterogeneity, which enhances the species diversity, especially a habitat contain rocks which provide micro-niches and moistures; long distance dispersal of a species depends on a product of dispersibility and the population size of species and species influencing by the climatic fluctuation (Abulfatih et al., 2001).

However, water source is a limiting factor in the productivity of Qatari desert and controlling the distribution of most native vegetation species. Types of vegetation species are being determined through water source as one of the characteristics where most arid or hyper arid are being determined through the minimal precipitation and harsh environment conditions, including drought (Abulfatih et al., 2001). In addition, the main soil variables that control the distribution of vegetation and spreading species group to different regions include soil texture, pH, and $CaCO_3$ (Al-safran,2014).

Western shorelines of Arabian Peninsula are recognized with halophytic plants, which are plants that can grow under saline conditions (Abulfatih, 2002). Moreover, halophytes exist in the Arabian Peninsula inland areas, where water is available. Similarly, in Qatar, halophytes commonly found in wetland and inland salt flats and along the coastal areas (Abulfatih, 2002). All plants root absorbs salts from the soil. Halophytes can tolerate high levels of soil salts. However, high internal sodium and chloride are toxic to plant tissues which can disorganize enzyme functions and metabolic process. Halophytes can

reduce this toxicity by, for example, storage of NaCl in their cells' vacuoles (Abulfatih, 2002). Succulent plants, which is another term of halophytic plants that are accumulate sodium and chloride in their tissues, have high level water storage. More soil salinity means the succulence of the plants become greater (Abulfatih, 2002). Some halophytes, when the salts accumulate in high level within the plant tissues, the leaves were possessed to secrete the excess salts, mainly sodium and chloride, to the outside from the glands in both sides of leaves, and finally, these crystals of salts were dropped to the soil or washed off by rain, dew or wind. An example of these type of plants is *Limonium axillare* (Abulfatih, 2002).

2.6 The Studied Plant Species

Amaranthaceae family consist of 175 genera with about 2000 species. The Amarenthaceae are annual or perennial plants, some are shrubs, vines, and small trees, but most are herbs (Mroczek, 2015). The crude extract encompasses flavonoids, essential oils, phenolic acids, triterpenes, and diterpenes. Amaranthaceae extracts showed significant antioxidant, antitumor, antibacterial, antiulcer, larvicidal, anti-inflammatory and analgesic activities (Mroczek, 2015). Most of the plants (*Aerva javanica, Salsola soda,* and *Suaeda vermiculata*) that are selected to carry out this research belong to Amaranthaceae. Lastly, *Limonium axillare* belongs to the succulent Plumbanginaceae family.

2.6.1 Aerva javanica (Burum. f.) Spreng: Family Amaranthaceae

About 28 species of *Aerva* genus exist around the world, but a limited number of species have medicinal value (Chawla, 2012). Among its species; *A. persica, A. lanata* and

A. javanica are of great value (Chawla, Chawla, Vasudeva, & Sharma, 2012). *A. javanica* is a perennial plant that is native to Africa and Asia and is distributed in various parts of the word (Arbab et al., 2016; Mufti et al., 2012). *A. javanica* is a densely dwarf shrub with a height of 15-80 cm. Its leaves are flat and alternate with short-petioled. This plant is common in Qatar; it grows on shallow rocky soils. The flowering period of *A. javanica* is from December to June. Its local name is Trif, Twaim, El Rawa (Batanouny, 1981).

Traditionally, this plant is used as a medicine for diuretic, demulcent, purgative, emetic and tinder, and as a decoction of areal parts, it is used to cure gum swelling and toothache (Arbab et al., 2016; Chawla et al., 2012). Also, its seeds are used for headache relief, and its flowers and leaves are used for wounds healing and joints inflammation (Arbab et al., 2016). In addition, Qatari Bedouin uses flowers head for pillows and stuffing saddles (Norton, 2009).

As mentioned in Chawla et al., (2012), Different types of *Aerva* species have major phytochemical constituents, which are several numbers of flavonol glycosides, and minor phytochemicals, which are β-cyanins, sterols, and carbohydrates. The flavonol glycosides that are reported in *A. javanica* species from the fresh aerial parts are Kaempferol-3-galactoside, 3-rhamnogalactoside, quercetin-3-galactoside, isorhamnetin-3-galactoside, 3-rhamnosyl-(1→6)-galactoside and 3-(p-coumaroyl)-rhamnogalactoside. In addition, the chrysoeriol, which is a flavonoid constituent, was isolated from aqueous and ethanolic extracts of *A. javanica*. Some sterols that are reported from this species are campesterol, sitosterol, 7-ergostenol, spinasterol, 7-stigmastenol, campestanol, and 22-stigmastenol. In addition, β- Sitosterol, triterpenoid α- and β-amyrin and pentadecanoic acid have been reported in GLC analysis of the unsaponifiable fraction of *A. javanica*. Other

phytochemicals have been reported in *A. javanica* species, which are arabinose, rhamnose, xylose, galactose, glucose, mannose, and mannitol (Chawla et al., 2012).

The flavonoidal phytochemicals that are isolated from aqueous and alcoholic extract of *A. javanica* presented significant antimicrobial activities against fungi, yeast and Gram-negative bacteria (Arbab et al., 2016; Chawla et al., 2012). Shad and colleagues (2016) reported that the aqueous methanolic fraction of *A. javanica* showed maximum activity against *Fusarium solani* in comparison with other strains of fungi. Also, it showed significant activity against *Candida albicans*, with negligible activity against *Trichophyton longifusus*. On another hand, a screening study done by Mufti and his team (2012) showed that *A. javanica* fractions do not have any significant antifungal activity against *Aspergillus flavus, A. fumigatus, A. niger* and *Fusarium solani*.

However, some *Aerva* species have been reported as an antioxidant plant (Chawla et al., 2012). *A. javanica* has been reported to have antioxidant activities that have come from the ethanolic extract of its aerial part (Arbab et al., 2016; Hashmi et al., 2017; Khan et al., 2012). Shad and colleagues (2017) reported that *A. javanica* showed considerable antioxidant activities, which are highest in hexane fractions followed by aqueous and methanol fractions.

2.6.2 Limonium axillare (Forssk.): Family Plumbanginaceae

The *Limonium* is a C3 halophytic genus with continuity of life cycle over the year, has about 350 species, and is diverse in the Mediterranean area, especially in Egypt, Arabian Peninsula, Qatar and Iran (Akhani, Malekmohammadi, Mahdavi, Gharibiyan, &

Chase, 2013). *Limonium axillare* belonging to Plumbanginaceae family is a short shrubby species with short flowering branches. Its habitat is hypersaline soils (Akhani et al., 2013). Its leaves are fleshy, petiolate with gland secreting salts. This plant is very common along the saline coasts of Qatar. The local name is Qataf, Gataf, Shleil (Batanouny, 1981). *L. axillare* butanol extract was distinguished to have superior antifungal activity (Mahasneh, 2002). The same study reported that *L. axillare* showed significant antifungal activity against *Aspergillus flavus, Candida albicans,* and yeast. It also exhibits antibacterial activity against Gram-positive and Gram-negative bacteria (Mahasneh, 2002).

2.6.3 Salsola soda L. Family Amaranthaceae

Salsola soda is a C4 plant species that belongs to succulent Amaranthaceae/ Chenopodiaceae family. It is a glabrous costal annual plant, with semi-terete, half-clasping, and 1-6 cm long leaves. Commonly, it found in the coastal salt marshes near Al-Khor (Batanouny, 1981). Traditionally, *Salsola* species are used as anticancer, anti-inflammatory, antihypertensive and antiulcer (Tundis et al., 2009). *S. soda* extracts have hypoglycemic activity by the inhibition of the enzyme α- amylase (Tundis et al., 2009). So far, according to my knowledge, there is no research about the antifungal and anticancer activities of *S. soda* species, particularly that is found in the state of Qatar.

2.6.4 Suaeda vermiculata (Forssek.) Family Amaranthaceae

Suaeda vermiculata, that belongs to Amaranthaceae family, is an edible halophytic plant with high tolerance to salinity that makes it capable of living in salt marshes

(Cybulska, Brudecki, Alassali, Thomsen, & Brown, 2014; Motamed, Bush, Rouzbahani, Karimi, & Mohammadipour, 2016). *S. vermiculata* is an intricately branched low shrub of dark green color. Its leaves are succulent, petioled and obtuse, that turns black after drying. It has sessile flowers with a cluster of 1-3 and with a fruiting perianth of 1 mm diameter. It is found in south-west Qatar in the littoral zone dominating a community at Abu Samra. The local names given to this plant are Suwweid, Tuwaim, Sha'aran, Girm (Batanouny, 1981). Therapeutically, *S. vermiculata* was used for inflammation, breathing complications and Jaundice (Motamed et al., 2016). Cybulska and his team (2014) reported that the water extract of *S. vermiculata* acts as hypoglycemic and hypolipidemic agents in rats. Also, it has reported that the shoot extracts contain a high content of polyphenols, including flavonoids that have the properties of free radical-scavenger (Cybulska et al., 2014). Polyphenols along with triterpenoids have a valuable affect in preventing cancer diseases (Cybulska et al., 2014).

2.7 Rationale

The observations described in the literature above form the rationale for this research. The research will consider or highlight on how the conventional medicine is being challenged with inefficacy of current anticancer drugs due to drug resistance phenomena, therefore, the need for new anticancer agents with better efficacy and lesser side effects. However, several studies have extensively examined antimicrobial and anticancer effects using phytochemical compounds. The present study will test the antimicrobial and anticancer effects of different selected Qatari plant species. So far, these plants collected

from Qatari arid environment, were not yet tested for its antimicrobial and anticancer effects.

2.8 Objectives

- Determine the effect of different plant extracts on BC cells proliferation, inhibition of cell growth and induction of BC cells death.
- Evaluate the effect of different plant extracts against several fungal species using two methods and calculating Minimum Inhibitory Concentration (MIC) for each extract

3. METHODOLOGY

3.1 Plant Materials

The plant samples were collected from Qatari environment (Table 1). *Suaeda vermiculata* (Figure 1A) and *Limonium Axillare* (Figure 1C) from North of Qatar, *Salsola soda* (Figure 1D) from Al-Khor coastal area, and *Aerva javanica* (Figure 1B) from Qatar University. The collected plants were kept in paper bags, inside ice box, until being brought to the laboratory. Plants were cut to separate aboveground biomass (stem and leaves) from belowground biomass (root). Plants were then allowed to dry under room temperature and direct air in a shaded place for about 2 weeks. If needed, especially with succulent plants, they were put into an oven for 3-4 days at 45^{o}C to make sure the whole plant was dried. Then, the plant parts were grinded using a grinder to become powdery and then kept in labeled bottles in a dry place in the refrigerator (4^{o}C).

Table 1
Selected Plant Species That are Used in The Study Research.

Plant Species	Family	Major Character	Type	Collecting Location	Local Name
A. javanica	Amaranthaceae	Non-succulent/ C4	Xerophyte	Qatar University	Trif, Twaim
L. axillare	Plumbaginaceae	Succulent/ C3	Psammophile	Al-Khor coastal area	Qataf, Shleil
S. soda	Amaranthaceae	Succulent/ C4	Hydrohalophyte	North of Qatar	-
S. vermiculata	Amaranthaceae	Succulent/ C4	Xerophyte	North of Qatar	Suwweid, Sha'aran, Girm

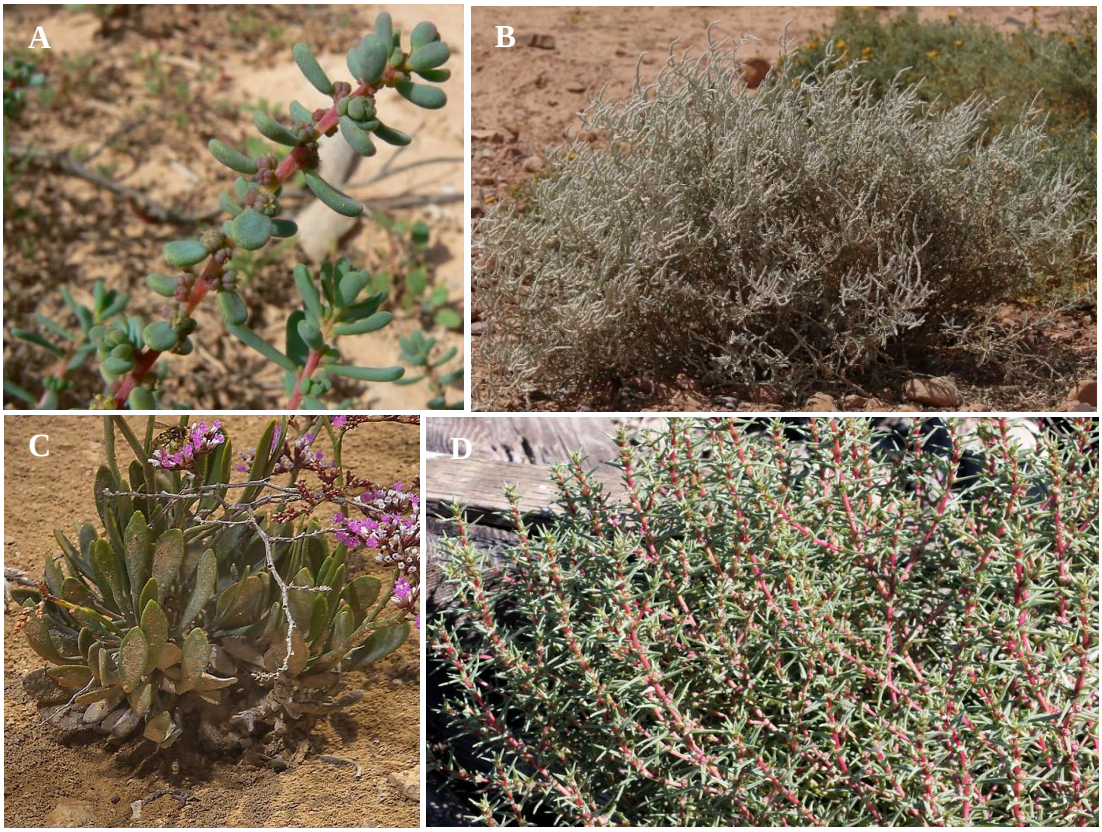

Figure 1. The selected native Qatari plant species; (A) *Suaeda vermiculata*, (B) *Aerva javanica*, (C) *Limonium axillare* and (D) *Salsola soda*.

3.2 Preparation of Crude Plant Extracts

3.2.1 Aqueous Extraction

3.2.1.1 For Antifungal Activity Tests

The aqueous extract has been obtained by boiling 20g of the ground air-dried material of plant, which has been put inside a cheese cloth bag, and immersed in 200 ml of sterilized distilled water for 8 hours in a shaker water bath at a (45^{o}C) temperature. The extract was taken and transferred into labeled glass Petri plates and allowed to evaporate in the oven at (45^{o}C) temperature to get gummy powdery materials, then kept in the

refrigerator at (4ºC) temperature. Solutions of different concentrations of the extract have been prepared for antifungal activity assays by dissolving 100 mg of extract in 10 ml of sterilized distilled water to obtain a final stock concentration of 10mg/ml. Then, the stock solution was diluted to 5 and 2.5 mg/ml concentrations.

3.2.1.2 For Cytotoxic Assay

For cytotoxicity assay, (300 mg) of extract was dissolved in 300 µl of Dulbecco's Modified Eagle Medium (DMEM) (1x, Gibco|Thermo Fisher Scientific) supplemented with 10% Fetal Bovine Serum (FBS, Sigma-Aldrich) and 1% penicillin (Sigma-Aldrich) to obtain stock concentration of 1mg/µl. Then, the stock solution was diluted by DMEM supplemented with 10% FBS and 1% penicillin to 500, 400, 300, 200, 100 and 75 µg/µl.

3.2.2 Organic Extraction

3.2.2.1 For Antifungal Activity Tests

Twenty grams of the air-dried powdered plant material have been put inside a cheese cloth bag and then immersed in 95% ethanol, methanol or acetone (2 times of 20 hours × 200 ml each time) in a shaker water path at (35ºC) temperature. After that, the extracted material was evaporated using a rotary evaporator and the powdered material was collected and then kept in the refrigerator (4ºC) temperature. Crude extract solutions of different concentrations have been prepared for antifungal activity assay by dissolving (0.1 g) weight amount of extracted material in (10 ml) dimetylsulfoxide (DMSO) to obtain a

final stock concentration of 10 mg/ml. Then, the stock solution was diluted by sterilized distilled water to 5 and 2.5 mg/ml concentrations.

3.2.2.2 For Cytotoxicity Assay

For cytotoxicity assay, (300 mg) of the extract was dissolved in (300 µl) of DMSO (4% volume of the total amount of solution) and DMEM supplemented with 10% FBS and 1% penicillin (96% volume of the total amount of solution) to obtain a stock concentration of 1mg/µl. Then, the stock solution was diluted by DMEM supplemented with 10% FBS and 1% penicillin to obtain concentrations of 500, 400, 300, 200, 100 and 75 µg/µl, and kept in a refrigerator at (4^{o}C) temperature.

3.3 Antifungal Activity Assay

Fungi Culture. Fungi were properly isolated from stock cultures that were isolated from Doha atmosphere and stored in Dr. Abu-Dieyeh laboratory and then grown on Potato dextrose agar (PDA) in the incubator at (25^{o}C) temperature for 3-5 days. The tested fungal species include the following: *Alternaria alternata, Cladosporium sphaerospermum, Fusarium oxysporum,* and *Botrytis cinerea*.

Agar Diffusion Method. The spore suspension was prepared using sterile distilled water and then serially diluted one hour prior to experimental work to reach a density of 1 x 10^{7} spore/ml (see Figure 2). Petri plates (7 cm diameter) have been used for antifungal assays. Different concentrations of plant extracts were prepared as mentioned above (section 3.2.). In each Petri plate, 1 ml of the selected plant extract have been mixed with

1 ml of selected fungal spore suspension and then poured 9 ml molten PDA (~ 40^{o}C temperature) supplemented with Agar-Agar (1 g/L). The Petri plates gently mix all of its content homogeneously. Control testing was prepared without plant extracts as a positive control and without spore suspension as a negative control. The Petri plates have been incubated for 4-5 days in the incubator at (25^{o}C) temperature (Baloairi, 2016). On each plate and after incubation, all colony forming units (CFU) were counted and recorded. Three replicates have been used for each treatment level, and the whole experiment was conducted twice for each organism, one with water extracts and one with ethanolic extracts.

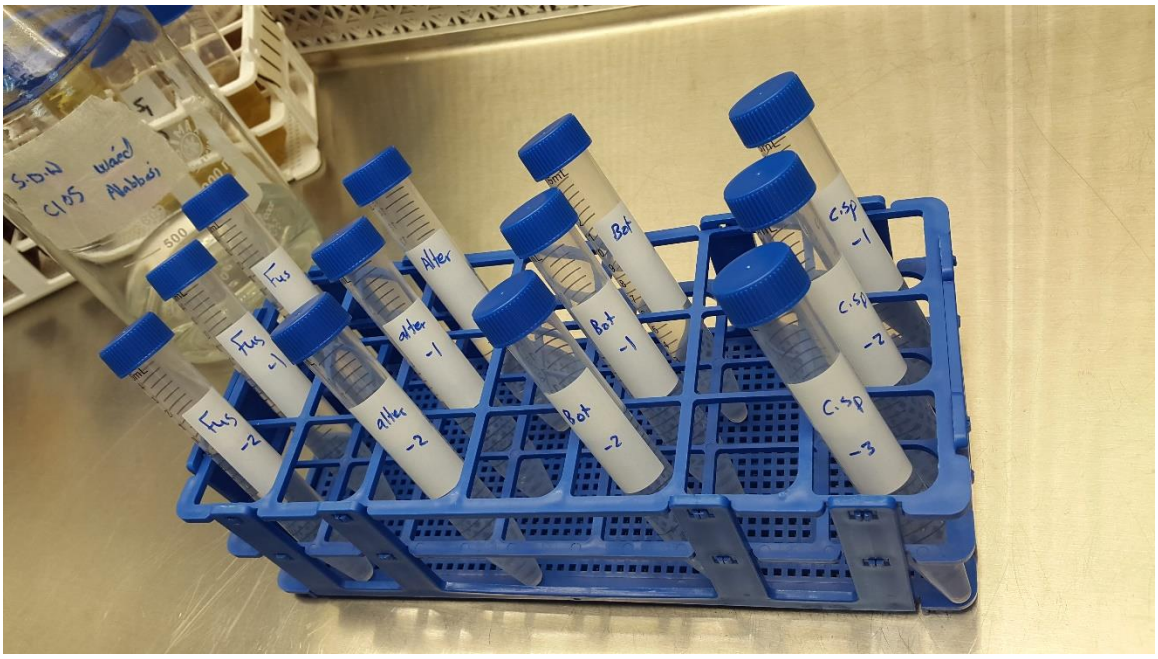

Figure 2. Spore suspension and dilutions of selected fungus species; *Alternaria alternate*, *Cladosporium sphaerospermum*, *Fusarium oxysporum*, and *Botrytis cinerea*

<u>*Poisoned Food Method*</u>. Disc of mycelia (7 mm diameter) has been cut from the marginal region of 6-10 days old pure culture of the selected fungus (see Figure 3) and then

were transferred to the center of Petri dish (7 cm diameter) containing PDA inoculated with different concentrations of 10, 5, 2.5, 1.25 and 0.75 mg/ml of plant extract (1 ml/dish). The positive control for this experiment was prepared without plant extracts, and the negative control was prepared without mycelia disc. Petri dishes have been incubated at 25°C. The antifungal effects were determined after 4 days by measuring the colony diameter along the two axes at right angles to each other using a Vernier caliper in mm. The fungal activity was calculated as percentage inhibition of radial mycelial growth (IMG %) using the formula: IMG (%) = [(dc - dt)/dc] x100, where dc is the radial mycelial growth measurements in control and where dt is the radial mycelial growth measurements in treated plates (Baloairi, 2016).

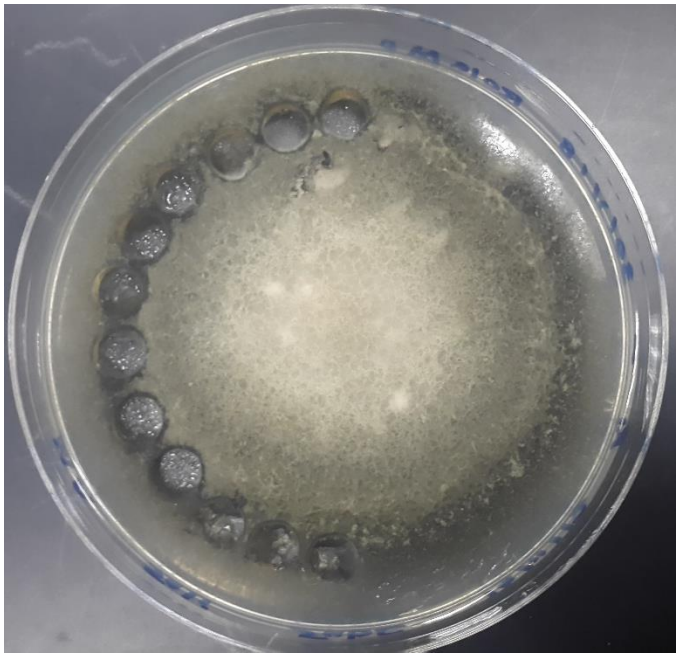

Figure 3. Poisoned food method in *Altrnaria alternata* fungus species by disc (7 cm diameter).

3.4. Cytotoxicity Activity Assay

Cell Culture. A breast cancer cell line, MCF-7, was cultured in DMEM medium supplemented with 10% FBS and 1% penicillin. Cells were incubated at 37°C in the presence of 5% CO_2 in a humidified chamber and allow to reach 80-90% confluence (see Figure 4).

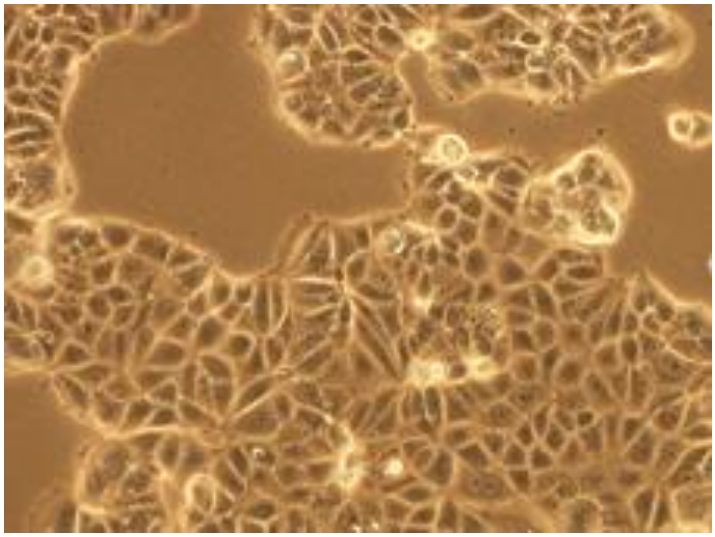

Figure 4. MCF-7 (breast) cancer cell line with confluence reach to 90%, under the compound microscope (40x). Retrieved form: http://tgrbio.com/cellular-models/mcf7-cells/

AlamarBlue® Cell Proliferation Assay. MCF-7 cell line was plated on clear bottom 96 well plates (5000 cells/well) and cultured in DMEM supplemented with 10% FBS and 1% penicillin (100 µl/well) for 24 hours and incubated at 5% CO_2, 37ºC in a humidified chamber, allowed the cells to attach (Figure 5). Then, the dilutions were cultured to each well (100 µl/well) and incubated for 48 hours, after removing of all the previous media. The solution of (550 µl) AlamarBlue® dye and (9.5 ml) DMEM medium supplemented with 10% FBS and 1% penicillin was added on all wells (100 µl/well) after the removal of all media and incubated for 2.5 hours in the dark humidified chamber with 37ºC temperature and 5% CO_2. Readings were taken at an absorbance of 570 nm wavelength using TECAN M200 Pro.

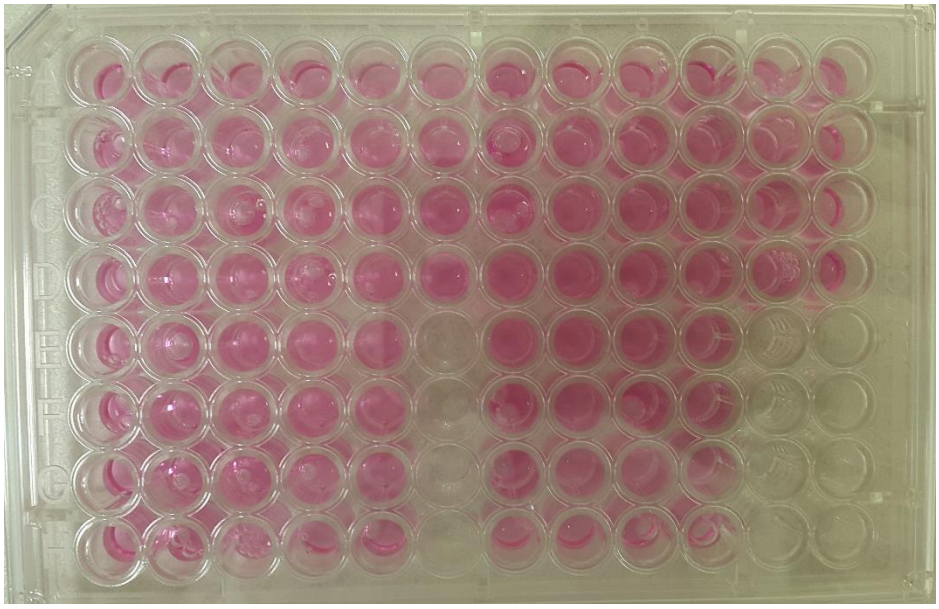

Figure 5. Platting of MCF-7 in 96-well plate (100µl/well).

Statistical Analysis. The student's t-test was applied at a significance level of $p \leq 0.05$ using Excel for data analysis, and n equals to ≥ 4.

4. RESULTS

4.1 Antifungal Activity Assays

4.1.1 Aqueous Crude Extraction of Plant Areal Parts

The results of this study showed that the aqueous extracts of the plant species, *Aerva javanica, Limonium axillare, Salsola soda* and *Suaeda vermiculata,* have no any significant antifungal activity against all four-fungal species tested; *Altrnaria alternata, Cladosporium sphaerospermum, Fusarium oxysporum,* and *Botrytis cinerea*. By poisoned food method, the antifungal activity was recorded by measuring the diameter of radial mycelial growth then calculating of the inhibition percentage of radial mycelial growth (% growth reduction) Table 2 shows the percentage of change in mycelial growth compared to the growth in the control. The results showed that the aqueous extracts exerted a very little effect on mycelial reduction of the studied fungi (Figure 6&7). The only significant reduction obtained from the aqueous of leaf extract of *A. javanica* on *F. oxysporum* which showed about 50% of inhibition.

When there is some reduction in fungal mycelia growth, the highest reduction in growth have been reported under the highest concentration of the extract (Table 2). Almost similar trend of results was reported after using the agar diffusion method instead of the poisoned food method (Table 3&4 and Figure 8). Interestingly in few cases, greater colony forming units have been reported in the presence of the aqueous plant extract, such as the colony forming units of *C. sphaerospermum* reached above 320 colonies at 5 and 2.5 mg/ml concentrations, while the control was 195 colonies in treatment with *S. soda*.

Table 2

The Change in Mycelial Growth of The Tested Fungal Species (%) As Influenced by Different Concentrations of Plant Crude Water Extracts Using Poisoned Food Method. (-) indicate more mycelia growth compared to control.

Plant Species	**Fungus**	**Extract Concentration (mg/ml)**		
		2.5	**5**	**10**
A. javanica	*F. oxysporum*	16	6.23	8.3
	A. alternata	1.69	-1.69*	-2.35
	C. sphaerospermum	16.94	-0.19	1.02
	B. cinerea	12.58	6.23	-5.82
L. axillare	*F. oxysporum*	-16.46	-25.44	-2.57
	A. alternata	2.09	-2.57	-3.05
	C. sphaerospermum	14.63	4.17	13.89
	B. cinerea	12.27	3.18	-13.89
S. soda	*F. oxysporum*	2.23	-0.24	3.93
	A. alternata	12.86	-0.82	-2.78
	C. sphaerospermum	4.18	13.86	6.38
	B. cinerea	14.50	20.20	-15.99
S. vermiculata	*F. oxysporum*	12.05	1.92	0.45
	A. alternata	8.52	2.30	3.67
	C. sphaerospermum	-24.75	-11.05	-69.51
	B. cinerea	4.86	-9.13	2.78

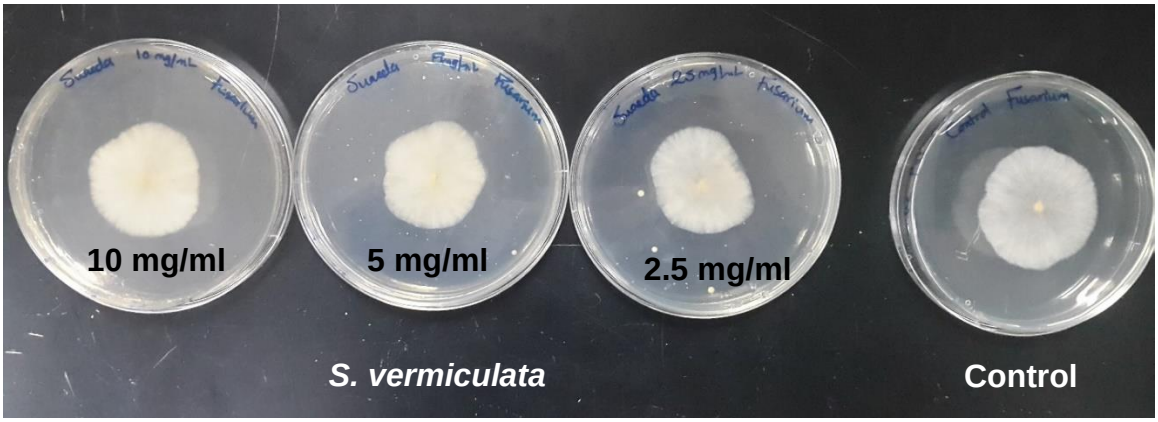

Figure 6. The growth of *F. oxysporum* as influenced by aqueous leaf extract of *S. vermiculata* by poisoned food diffusion method.

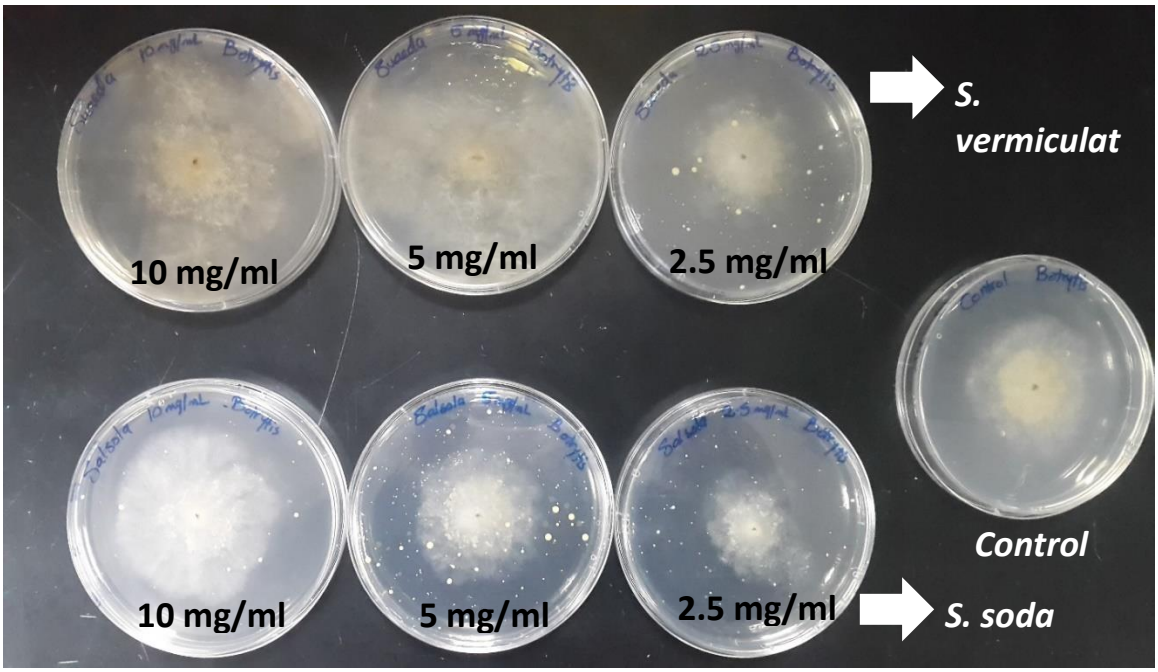

Figure 7. The growth of *B. cinerea* as influenced by aqueous extracts of *S. soda* and *S. vermiculata* by poisoned food diffusion method.

Table 3

Colony Forming Units (CFU) of Different Fungal Species as Influenced by Different Concentrations of Plant Crude Aqueous Extracts Using Agar Diffusion Method.

Plant Species	Fungus Species	Extract Concentration (mg/ml)			
		control	2.5	5	10
A. javanica	*F. oxysporum*	195	105	104.33	92.33
	A. alternata	18	20.67	21.67	23
	C. sphaerospermum	70.67	75.33	71.33	68.67
	B. cinerea	70	83.33	84.67	85.67
L. axillare	*F. oxysporum*	195	168.67	177.67	188.67
	A. alternata	-	-	-	-
	C. sphaerospermum	198	180.67	176	214.67
	B. cinerea	70	67.67	80.33	92.33
S. soda	*F. oxysporum*	195	195.67	193	192.33
	A. alternata	18	20.67	19.67	20.67
	C. sphaerospermum	195	325	354.67	172
	B. cinerea	70	63	65	74.67
S. vermiculata	*F. oxysporum*	195	181.67	190.33	185.5
	A. alternata	23	32	33	42.33
	C. sphaerospermum	198	439	332	654.67
	B. cinerea	70	85.67	90.67	90.33

Table 4

% Of Inhibition of The Colony Forming Units (CFU) of Different Fungal Species as Influenced by Different Concentrations of Plant Crude Aqueous Extracts Using Agar Diffusion Method. (-) Indicate More Mycelia Growth Compared to Control.

Plant Species	**Fungus**	**Extract Concentration (mg/ml)**		
		2.5	**5**	**10**
A. javanica	*F. oxysporum*	46.15	46.5	52.65
	A. alternata	-14.81*	-20.37	-27.78
	C. sphaerospermum	-6.60	-0.94	2.83
	B. cinerea	-19.05	-20.95	-22.38
L. axillare	*F. oxysporum*	13.50	8.89	3.25
	A. alternata	-	-	-
	C. sphaerospermum	8.75	11.11	-8.42
	B. cinerea	3.33	-14.76	-31.90
S. soda	*F. oxysporum*	-0.34	1.03	1.37
	A. alternata	-14.81	-9.26	-14.81
	C. sphaerospermum	-66.67	-81.88	11.79
	B. cinerea	10	7.14	-6.67
S. vermiculata	*F. oxysporum*	6.84	2.39	4.87
	A. alternata	-	-252.17	-84.06
	C. sphaerospermum	-121.72	-67.68	-230.64
	B. cinerea	-22.38	-29.52	-29.05

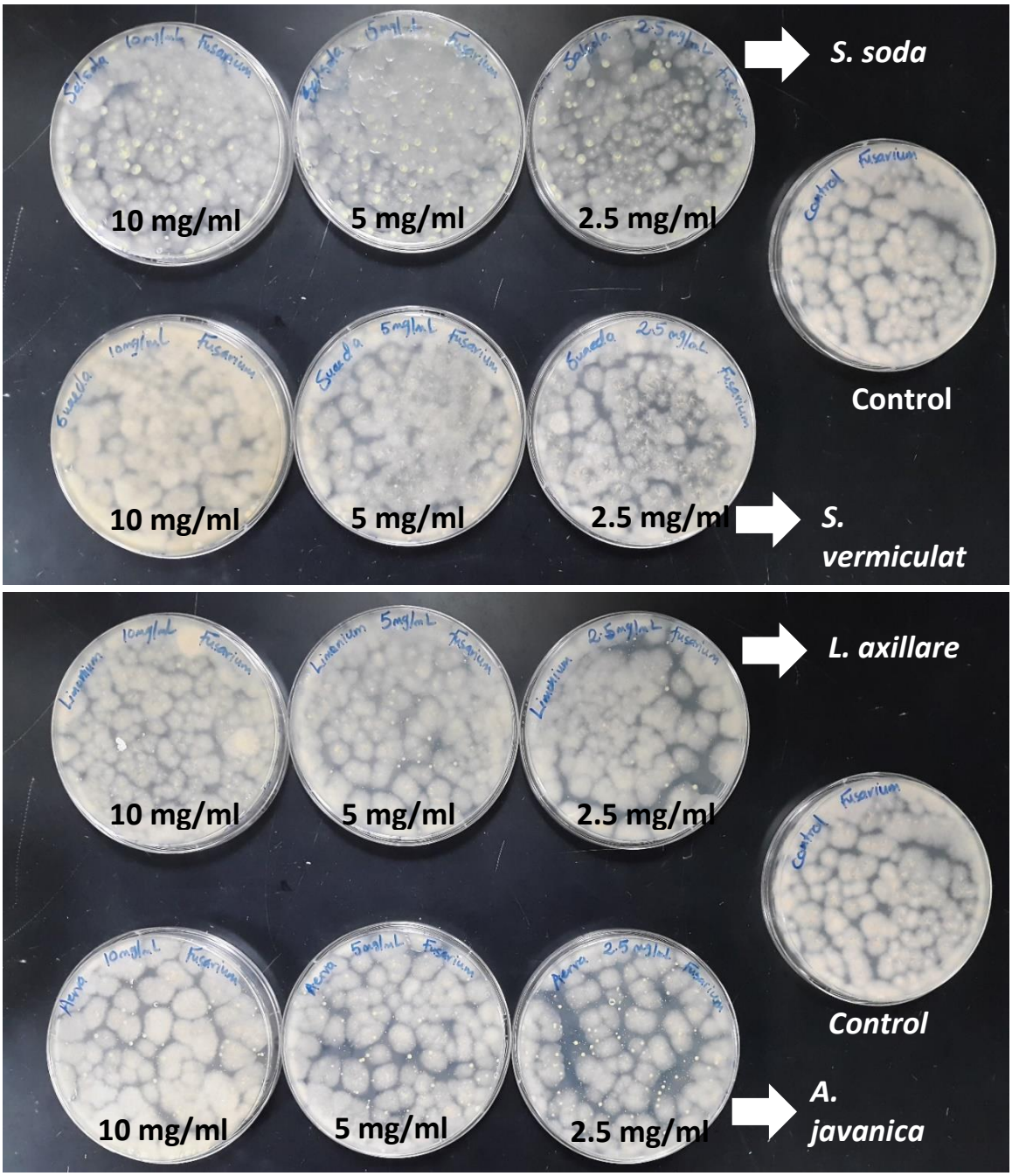

Figure 8. The growth of *F. oxysporum* as influenced by aqueous leaf extracts of (top) *S. soda*, *S. vermiculata*, (bottom) *A. javanica* and *L. axillare* by agar diffusion method.

4.1.2 Ethanolic Crude Extracts of Areal plant parts

The results of the current study showed that the ethanolic extracts of the plant species, *A. javanica, L. axillare, S. soda* and *S. vermiculata*, have a strong significant antifungal activity against all four-fungal species studied; *Alternaria alternata, Cladosporium sphaerospermum, Fusarium oxysporum,* and *Botrytis cinerea*. According to Table 5, the assessment of fungal growth reduction indicated that no fungal growth has been reported at different concentrations from different plants (Figures 9,10,11&12). The results have been confirmed by repeating the experiment using agar diffusion method instead of poisoned food method (Table 6&7) (Figures 13, 14 &15).

Table 5

The change in mycelial growth of the tested fungal species (%) as influenced by different concentrations of plant crude ethanol extracts using poisoned food method.

Plant Species	Fungus	Extract Concentration (mg/ml)		
		2.5	**5**	**10**
A. javanica	*F. oxysporum*	8.12	29.13	100
	A. alternata	37.72	66.34	100
	C. sphaerospermum	13.44	24.16	100
	B. cinerea	70.30	100	100
L. axillare	*F. oxysporum*	43.04	81.08	100
	A. alternata	62.59	100	100
	C. sphaerospermum	18.91	100	100
	B. cinerea	100	100	100
S. soda	*F. oxysporum*	11.97	32.62	100
	A. alternata	53.38	58.15	100
	C. sphaerospermum	17.61	24.30	100
	B. cinerea	75.32	100	100
S. vermiculata	*F. oxysporum*	37.79	75.23	100
	A. alternata	61.78	100	100
	C. sphaerospermum	18.86	100	100
	B. cinerea	100	100	100

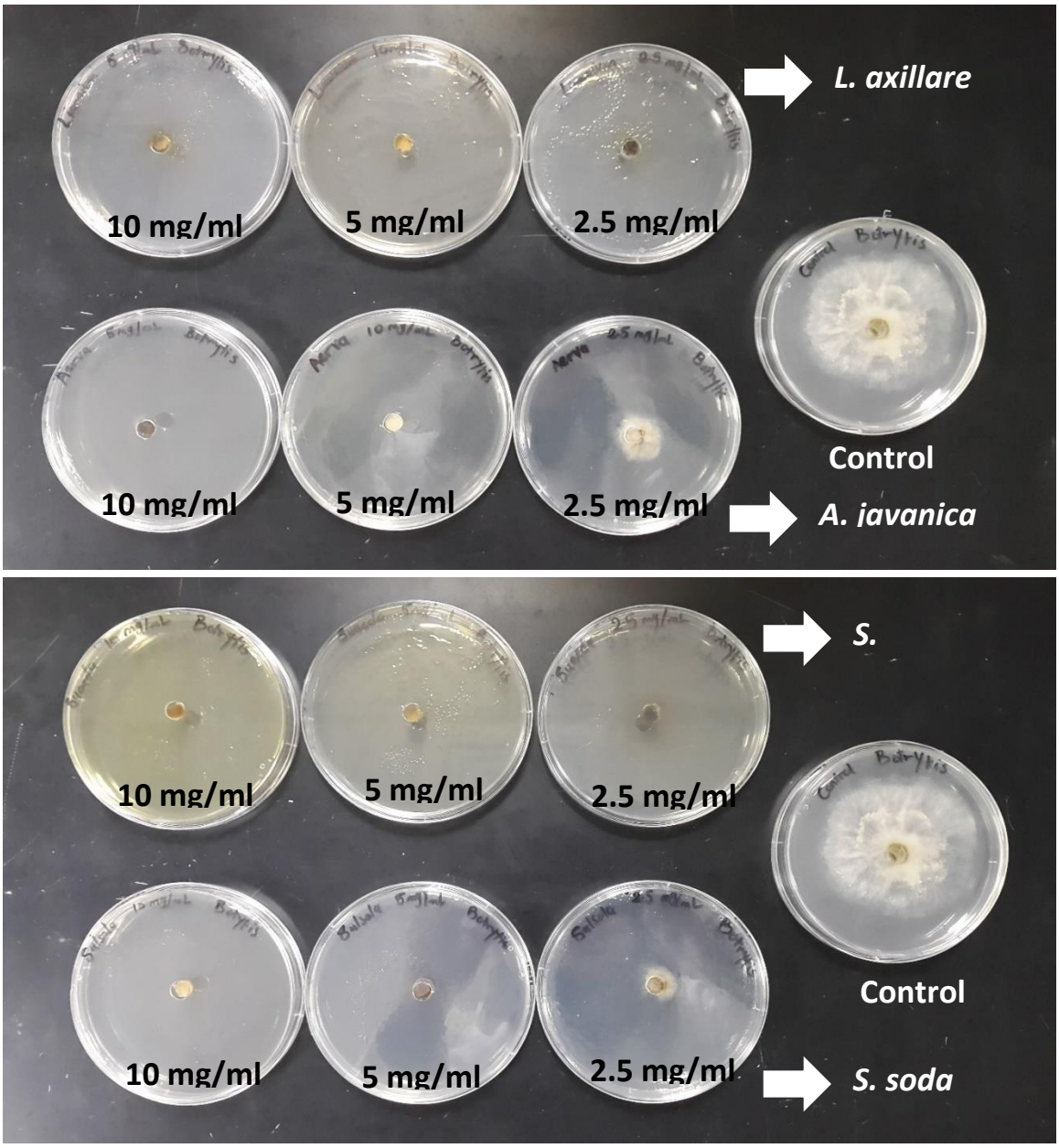

Figure 9. The growth of *B. cinerea* as influenced by ethanolic extracts of *A. javanica*, *L. axillare*, *S. soda* and *S. vermiculata* by poisoned food method. The picture shows the fungal disc did not grow at especially high concentration indicating to 100% inhibition in comparing to the control.

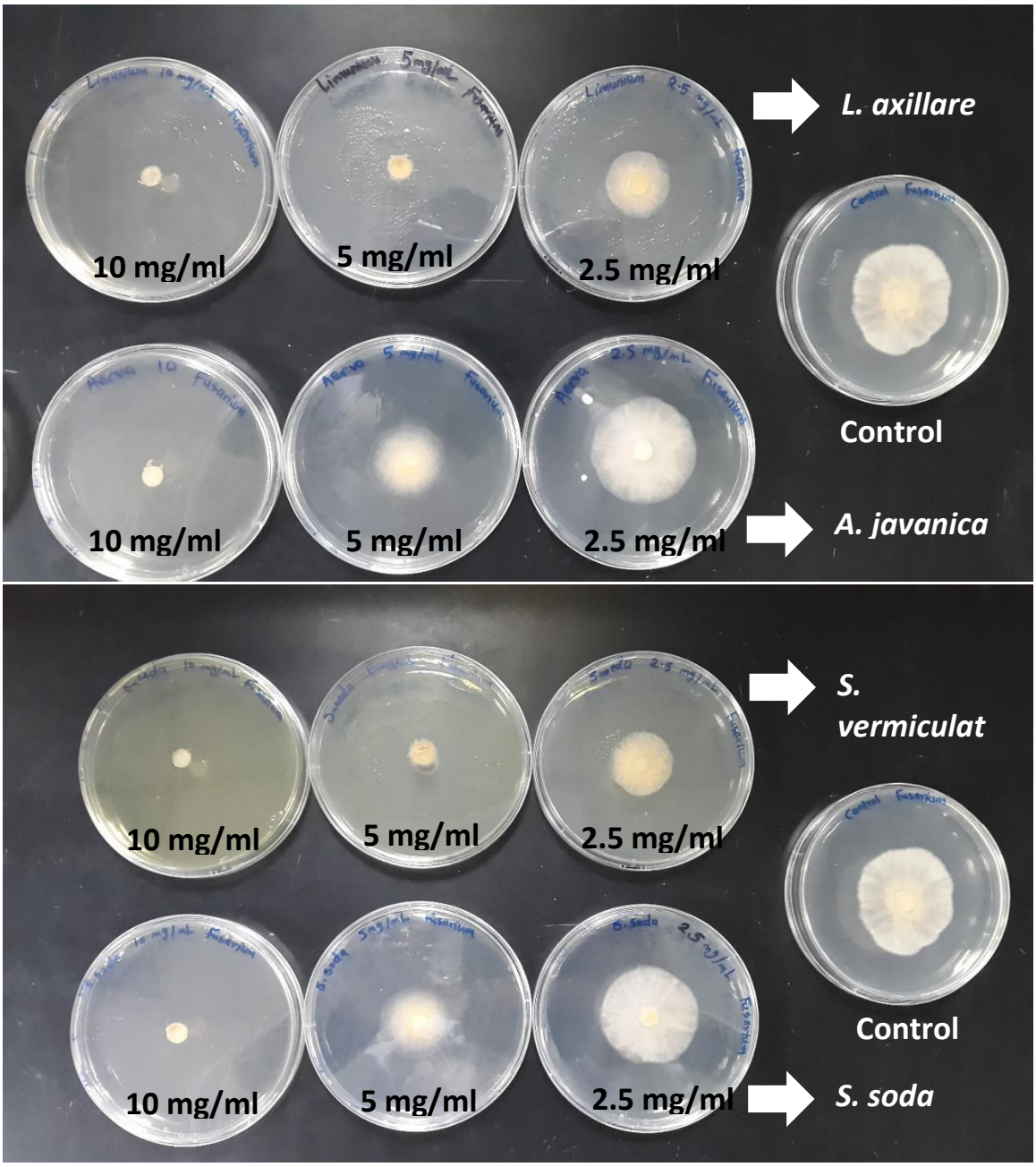

Figure 10. Fig: The growth of *F. oxysporum* as influenced by ethanolic extracts of *A. javanica*, *L. axillare*, *S. soda* and *S. vermiculata* by poisoned food method. The picture shows the fungal disc did not grow at especially high concentration indicating to 100% inhibition in comparing to the control.

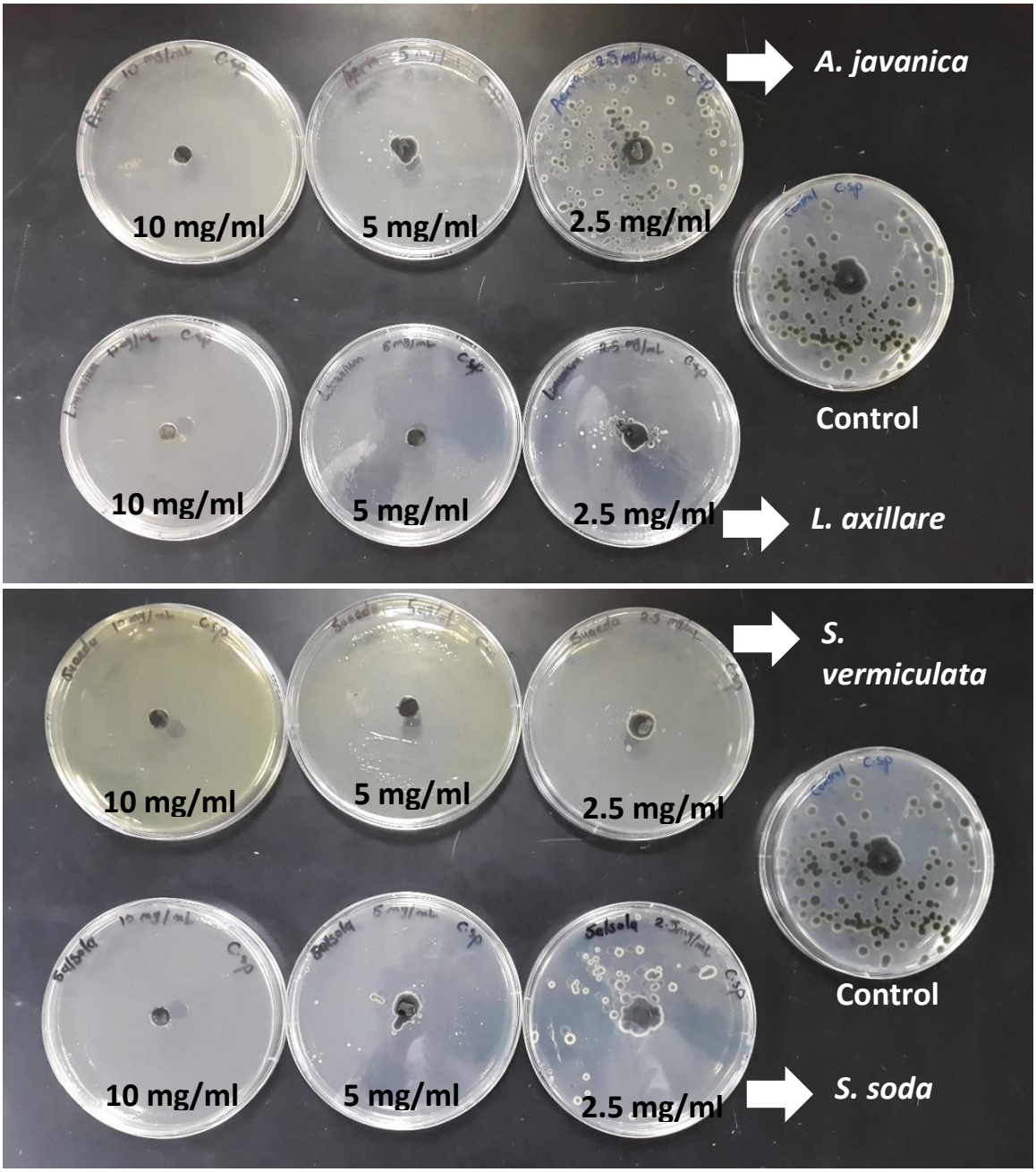

Figure 11. The growth of *C. sphaerospermum* as influenced by ethanolic extracts of *A. javanica, L. axillare, S. soda* and *S. vermiculata* by poisoned food method. The picture shows the fungal disc did not grow at especially high concentration indicating to 100% inhibition in comparing to the control.

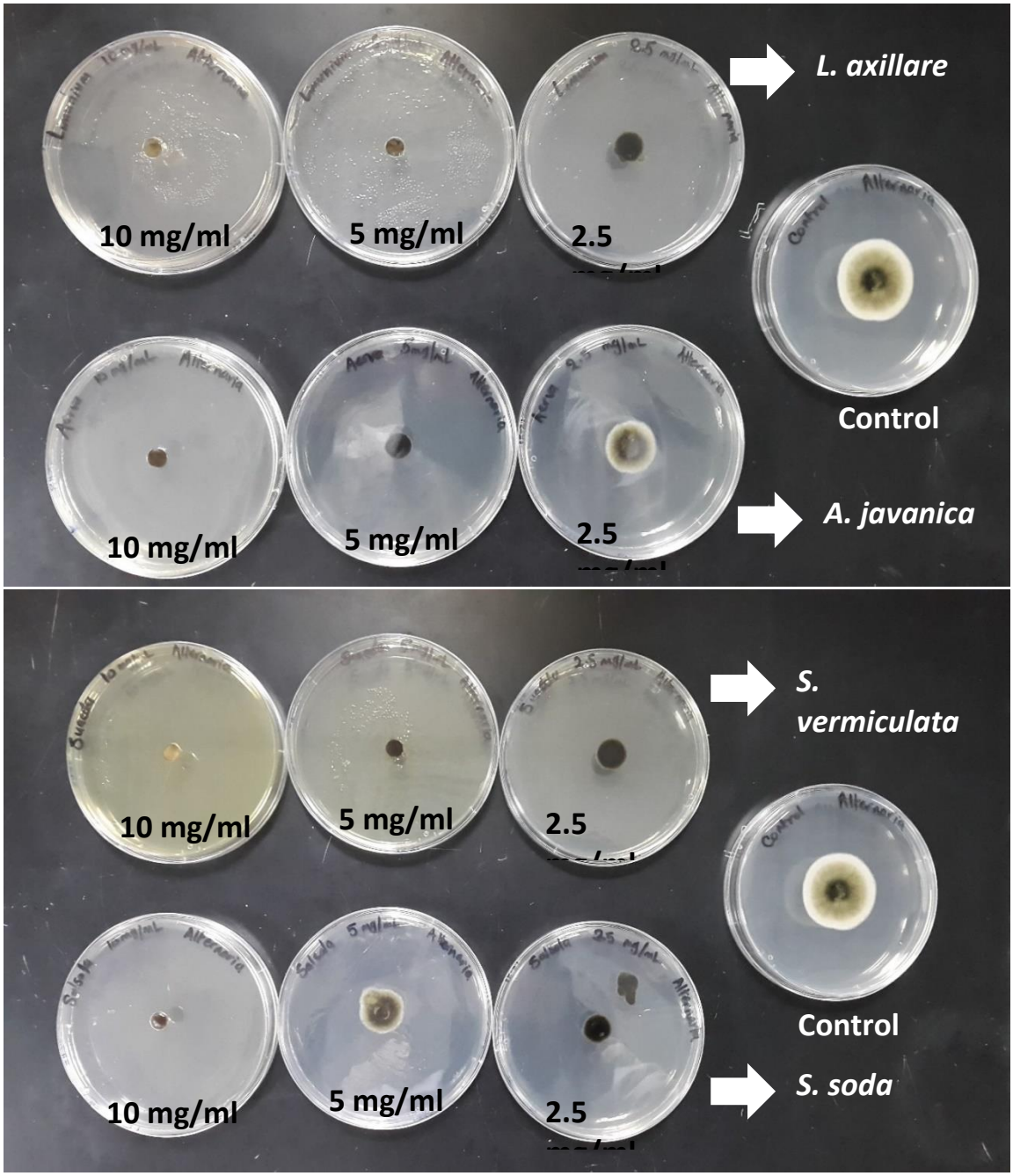

Figure 12. The growth of *A. alternata* as influenced by ethanolic extracts of *A. javanica, L. axillare, S. soda* and *S. vermiculata* by poisoned food method. The picture shows the fungal disc did not grow at especially high concentration indicating to 100% inhibition in comparing to the control.

Table 6

Colony Forming Units (CFU) of Different Fungal Species as Influenced by Different Concentrations of Plant Crude Ethanol Extracts Using Agar Diffusion Method.

Plant species	**Fungal species**	**Extract Concentration (mg/ml)**			
		Control	**2.5**	**5**	**10**
A. javanica	*F. oxysporum*	35.33	51.67	32.67	0
	A. alternata	11	9	0	0
	C. sphaerospermum	8.67	5.67	0	0
L. axillare	*F. oxysporum*	54	56	63	0
	A. alternata	11	19	0	0
	C. sphaerospermum	87	74	51	0
S. soda	*F. oxysporum*	35.33	37	38.67	0
	A. alternata	17.33	21.67	23.5	0
	C. sphaerospermum	8.67	3.67	0	0
S. vermiculata	*F. oxysporum*	35.33	40	32	0
	A. alternata	11	19	0	0
	C. sphaerospermum	8.67	3.33	0	0

Table 7

% of Inhibition of The Colony Forming Units (CFU) of Different Fungal Species os Influenced by Different Concentrations of Plant Crude Ethanol Extracts Using Agar Diffusion Method. (-) Indicate More Spore Colonies Growth Compared to Control.

Plant species	**Fungal species**	**Extract Concentration (mg/ml)**		
		2.5	**5**	**10**
A. javanica	*F. oxysporum*	-46.25*	7.53	100
	B. alternata	18.18	100	100
	C. sphaerospermum	34.60	100	100
L. axillare	*F. oxysporum*	-3.70	-16.67	100
	B. alternata	-72.73	100	100
	C. sphaerospermum	14.94	41.38	100
S. soda	*F. oxysporum*	-4.73	-9.45	100
	B. alternata	-25.04	-35.60	100
	C. sphaerospermum	57.67	100	100
S. vermiculata	*F. oxysporum*	-13.22	9.43	100
	B. alternata	-72.73	100	100
	C. sphaerospermum	61.59	100	100

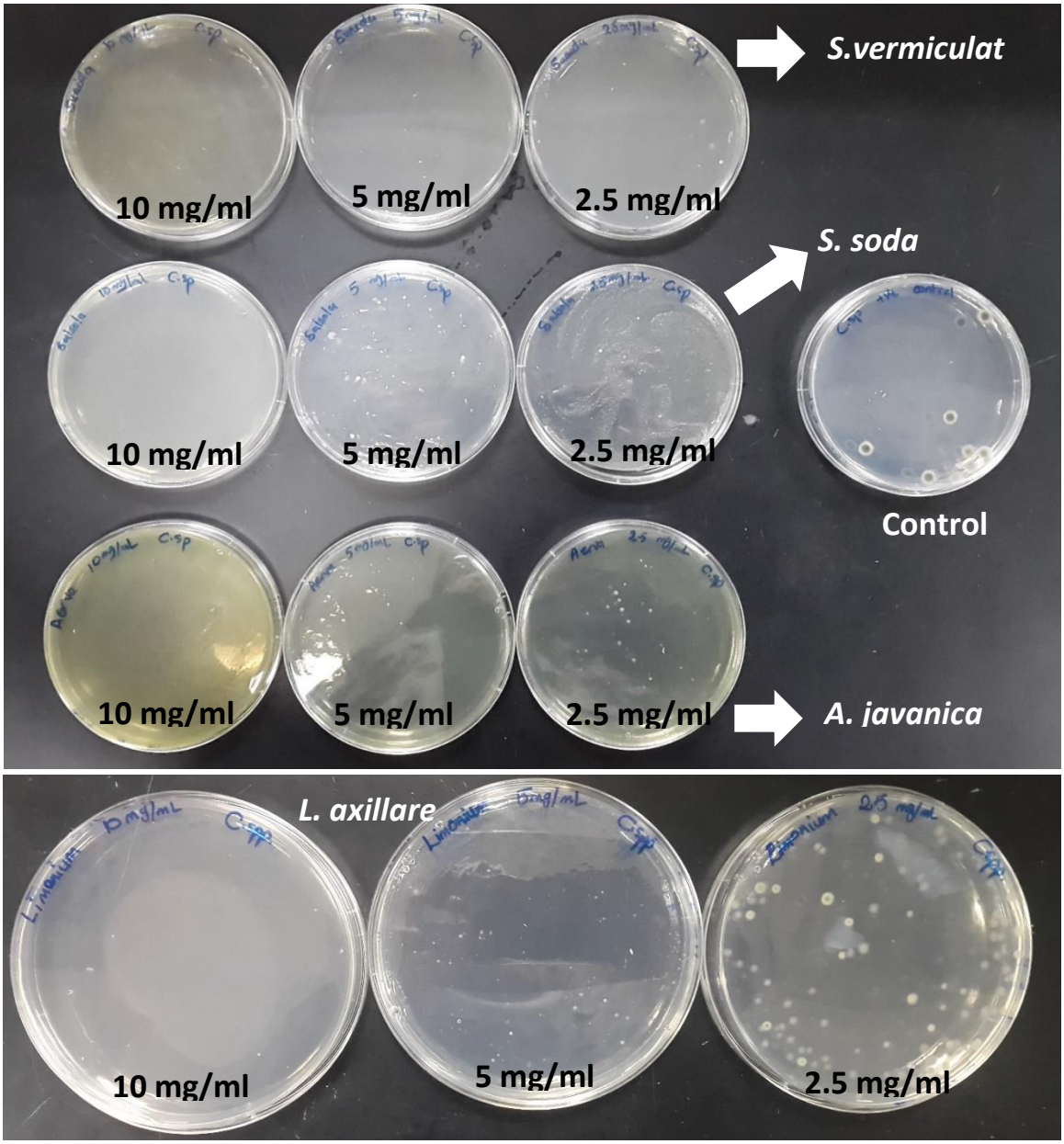

Figure 13. The growth of *C. sphaerospermum* as influenced by ethanolic extracts of *A. javanica, L. axillare, S. soda* and *S. vermiculata* by agar diffusion method. The picture shows the colony forming units (CFU) did not grow at especially high concentration indicating to 100% inhibition in comparing to the control. And even at the low concentration there is a growth, but the diameter of each colony was smaller than the control's colony, which is mean the inhibition process is continuing.

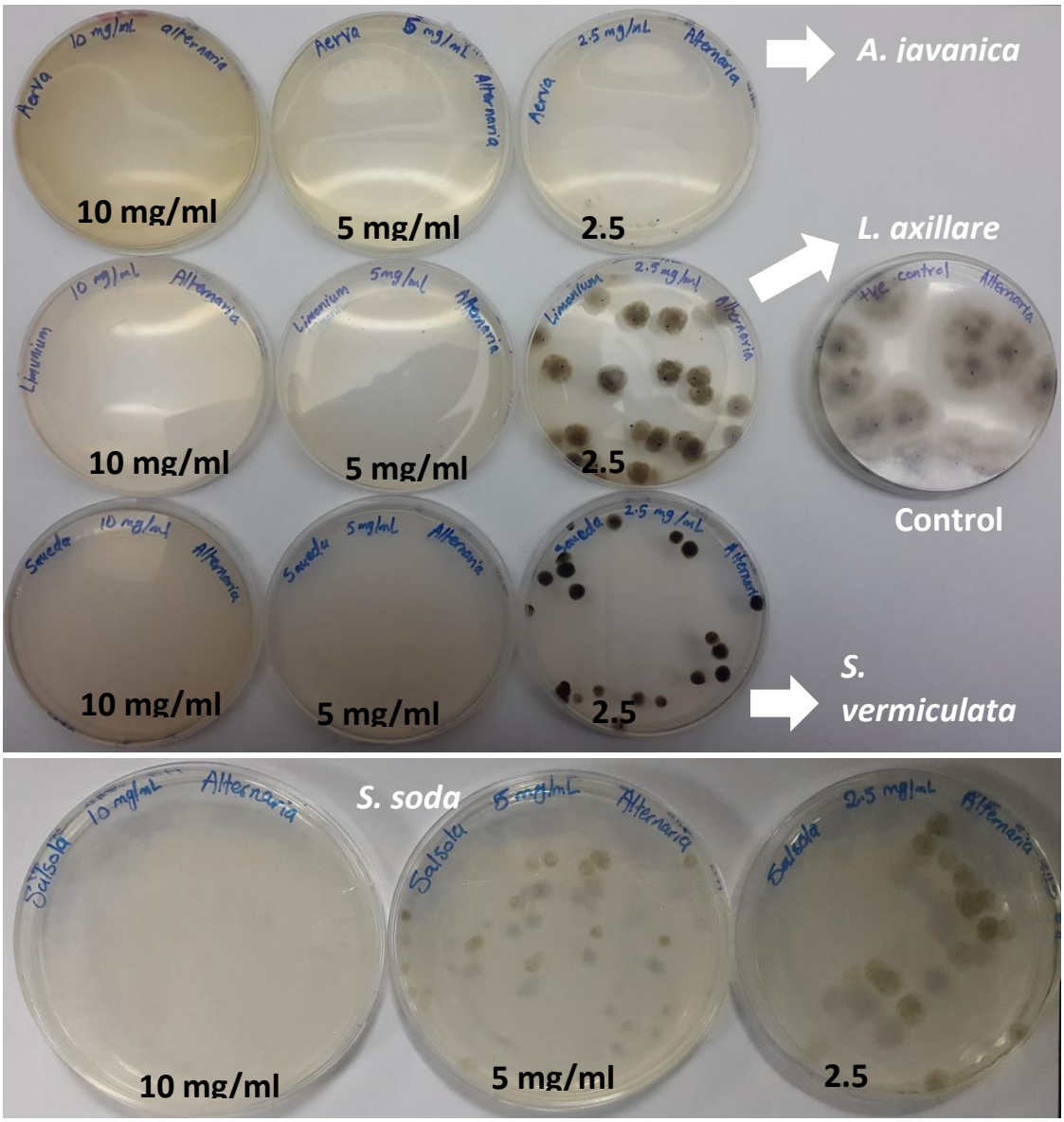

Figure 14. The growth of *A. alternata* as influenced by ethanolic extracts of *A. javanica*, *L. axillare*, *S. soda* and *S. vermiculata* by agar diffusion method. The picture shows the colony forming units (CFU) did not grow at especially high concentration indicating to 100% inhibition in comparing to the control. And even at the low concentration there is a growth, but the diameter of each colony was smaller than the control's colony, which is mean the inhibition process is continuing.

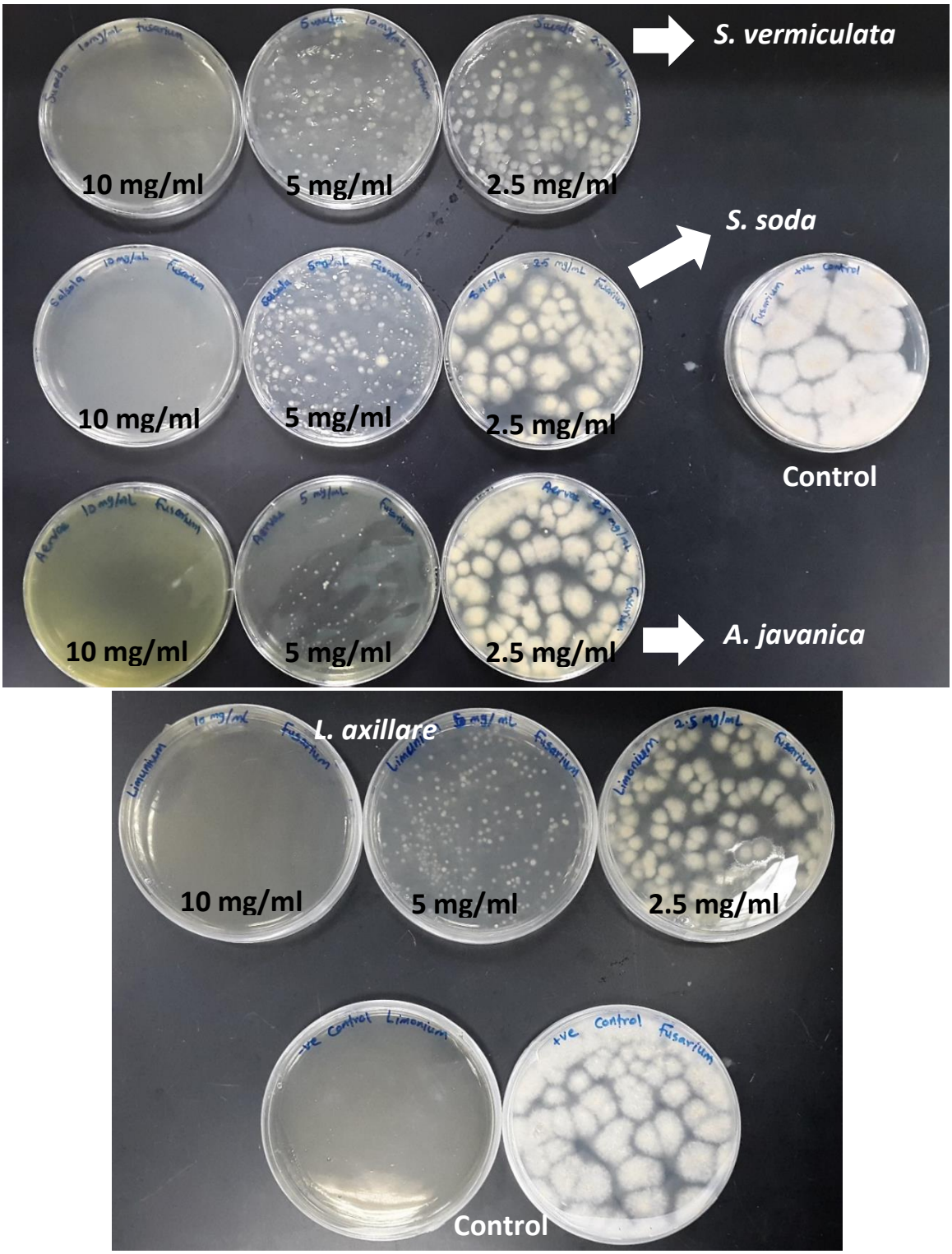

Figure 15. The growth of *F. oxysporum* as influenced by ethanolic extracts of *A. javanica*, *L. axillare*, *S. soda* and *S. vermiculata* by agar diffusion method. The picture shows the colony forming units (CFU) did not grow at especially high concentration indicating to 100% inhibition in comparing to the control. And even at the low concentration there is a growth, but the diameter of each colony was smaller than the control's colony, which is mean the inhibition process is continuing.

Table 8

The Lowest Concentration f Ethanolic Leaf Extracts of The Studied Plant Species That Totally Inhibit Fungal Growth Using Two Different Assay Methods.

Fungus Species	Plant Species	Poisoned Food (mg/ml)	Agar Diffusion (mg/ml)
A. alternata	*A. javanica*	10	5
	L. axillare	5	5
	S. soda	10	10
	S. vermiculata	5	5
F. oxysporum	*A. javanica*	10	10
	L. axillare	10	10
	S. soda	10	10
	S. vermiculata	10	10
C. sphaerospermum	*A. javanica*	10	5
	L. axillare	5	10
	S. soda	10	5
	S. vermiculata	5	5
B. cinerea	*A. javanica*	-	5
	L. axillare	-	2.5
	S. soda	-	5
	S. vermiculata	-	2.5

4.2 Anticancer Activity Assay

The AlamarBlue® cell proliferation assay used in this study to assess cell growth versus cell death uses AlamarBlue®. Based on the interaction of living cells with blue colored resazurin, which is the active constituent of AlamarBlue® reagent, it is converted into the red colored resorufin, that lead to increased fluorescence/absorbance and change the color of cells' media to pink. To determine the effect of the crude extract from each plant on MCF-7 cell proliferation, we used various concentrations of the crude extract ranging from 100 to 400 µg/µl. Non-treated cells and cells treated with growth media containing the same volume of Ethanol used to dissolve the extract powder were used as controls in the experiments.

Salsola soda methanolic and water extracts did not exhibit any significant activity against MCF-7 cell line (student's t-test at $p \leq 0.05$) in comparison to the control. However, methanol extract, the absorbance of 100, 200 and 300 µg/µl treatment concentrations were 0.61, 0.61 and 0.698 nm, respectively. While in the water extract, the absorbance of 100, 200, 300 and 400 µg/µl treatment concentrations were 0.62, 0.610, 0.698 and 0.68 nm, respectively. We observed the totally changing in surrounding media color to the pink after AlamarBlue® assay (Table 9).

Limonium axillare methanolic extract has been shown the similar effects to the *S. soda* species with no significant effect on MCF-7. The absorbance corresponding to the concentrations 70, 100, 200 and 300 µg/µl treatment concentrations were 0.612, 0.59, 0.65 and 0.68 nm, respectively (Table 9).

Although, *A. javanica* methanolic extract showed a blue color of the media at a concentration of 400 µg/µl indicating cell death (Table 9), t-test shows no significance. The absorbance values corresponding to the concentrations 70, 100, 200, 300 and 400 µg/µl treatment concentrations were 0.59, 0.53, 0.56, 0.52 and 0.498 nm, respectively.

However, *Suaeda vermiculata* species exhibited a significant anti-proliferative activity by both water and methanolic extracts, particularly at concentration 400 µg/µl concentration (p= 0.049) (Table 9).

Table 9

Absorbance (nm) After 2.5 Hours For MCF-7 (Breast) Cancer Cell Line Influenced by Different Concentrations (µg/µl) of Different Crude Plant Extracts, Assayed With Alamarblue® Dye. Values Are Expressed as Means±SD (n≥4). [a] Student t-Test (p≤0.05).

Plant Species	Extract Type	Concentration (µg/µl)				
		Non-treated	**100**	**200**	**300**	**400**
L. axillare	MeOH	0.61± 0.07[a]	0.59± 0.02	0.65± 0.10	0.68± 0.07	-
A. javanica	MeOH	0.49± 0.01	0.53± 0.02	0.56± 0.02	0.52± 0.02	0.50± 0.06
S. soda	Water	0.57± 0.09	0.62± 0.02	0.61± 0.05	0.70± 0.17	0.68± 0.17
	MeOH	0.61± 0.07	0.61± 0.04	0.61± 0.05	0.70± 0.06	-
S. vermiculata	Water	0.57± 0.09	0.67± 0.04	0.75± 0.03	0.73± 0.02	0.73± 0.02
	MeOH	0.62± 0.08	0.63± 0.04	0.75± 0.14	0.71± 0.1	0.71± 0.06

5. DISCUSSION

5.1 Antifungal Activity Assays

Several studies reported the antimicrobial activities of different plant parts from various herb species. The extraction of active constituents of plants is largely dependent on the solvent type that used in the extraction procedure (Kalidindi et al., 2015). Alcohol is a general solvent, in which it can deal with the extraction completely with a variety of compounds polarities (Evans, 1996). The present results can further indicate that the phytochemical constituents, either polar or non-polar, can be more efficient through the organic solvent medium. As we can see from the results above, the water extracts showed much lesser effect than the ethanolic extracts, this might be due to the dissolving efficiency of the active constituents in alcoholic solvent being better than in water.

Results of the antifungal activity assay have shown that the ethanolic extracts are very effective on fungal growth even under the lowest concentration. Ethanolic extracts of *Aera javanica, Limonum axillare, Salsola soda* and *Suaeda vermiculata* showed their inhibitory effects against the four types of fungi; *Altrnaria alternata, Cladosporium sphaerospermum, Fusarium oxysporum,* and *Botrytis cinerea* at a concentration of 10 and/or 5 mg/ml in both methods. These fungi have negative commercially effects on plant crops as well as negative effects on the human health. *A. alternata* can infect the human by causing respiratory tract infections, and it is also known to cause the leaf spot for plants (Kalidindi et al., 2015). *C. sphaerospermum* is the main reason to cause spoilage for fruit and vegetables and cause allergy and occasionally causes phaeohyphomycosis diseases in humans (Yew et al., 2016). While the *F. oxysporum,* is known to cause wilt of plant seedlings and fruits (Doohan, 2011), *B. cinerea* , which is a very well-known fungal pathogen, can infect the plant causing grey mold disease especially in tomato (Bednarek, 2014).

At 10 mg/ml concentration, *Aerva javanica* can kill all fungal spores and totally inhibit the mycelial growth in all tested fungal species: *A. alternata, C. sphaerospermum, F. oxysporum,* and *B. cinerea*. The effects of *A. javanica* on *B. cinerea* is strong enough to kill spores and prevent mycelial growth at the lowest concentration 5 mg/ml in comparable with *A. alternata*, which reached about 66% of mycelia inhibition. However, the 5 and 2.5 mg/ml concentrations have little inhibitory effects against *F. oxysporum* and *C. sphaerospermum*. In addition, in the agar diffusion method, the little effects of the low concentration on spore inhibition was compensated by the diameter of each colony, which is an indicator for continuing of inhibition process. Also, water extract of *A. javanica* have a significant effect on *F. oxysporum* at all concentrations by 50% mycelia growth inhibition. Generally, alcoholic extracts areal parts of *Aerva* species, particularly *A. javanica,* have valuable effects against fungi, yeast and bacteria, this is due to their richness in flavonoids phytochemicals, which play strong roles in antimicrobial and cytotoxic process (Chawla, Chawla, Vasudeva, & Sharma, 2012).

Limonium axillare had shown the strongest antifungal activities amongst the studied plants. It can totally inhibit mycelial growth of *A. alternata and C. sphaerospermum* with 10 and 5 mg/ml, respectively. Also, it can inhibit the growth of *F. oxysporum* mycelia by 80% at 5 mg/ml, and by 43% at 2.5 mg/ml. The *B. cinerea* fungus was completely inhibited by *L. axillare* (100% of mycelia growth inhibition) at a lower concentration of 5 mg/ml. However, in agar diffusion method, the number of colonies obtained at 5 mg/ml is almost the same as the untreated control, the inhibition process is resuming with the very low size of any grown colony and no sporulation in comparison with the positive control. Several studies showed that many species of *Limonium* have potent antifungal activity against several types of fungi including *Candida* species, the authors attributed this effect to be due to flavonoids and phenolic content of this plant (Gadetskaya et al., 2016; Liu et al., 2016).

Suaeda vermiculata species have very similar results like *L. axillare*. It showed a total inhibition of mycelial growth of all fungi studied. The effect was performed at 10 and 5 mg/ml concentrations of ethanolic leaf extracts. Additionally, exerted 60% inhibition of *A. alternata*, 37% inhibition of *F. oxysporum* and 19% inhibition of *C.*

sphaerospermum at the lowest concentration (2.5 mg/ml). Therapeutically, organic extract of *S. vermiculata* shoot part reported to has a high content of polyphenols, including flavonoids, which are a valuable compound for antifungal property (Cybulska, Brudecki, Alassali, Thomsen, & Brown, 2014; Motamed, Bush, Rouzbahani, Karimi, & Mohammadipour, 2016). Studies on different *Suaeda* species; such as *S. maritima* and *S. iranshahrii*, reported that these species have antimicrobial activities due to their content of flavonoids and essential oils (Nayak et al., 2018)

Also, *Salsola soda* has a totally mycelia growth inhibitory effect at 10 mg/ml concentration of ethanolic extract against all fungus studied. It has inhibitory growth of 50-60% against *A. alternata*, about 20% inhibition against *C. sphaerospermum* and 32% and 12% inhibition against *F. oxysporum* by at 5 and 2.5 mg/ml concentrations, respectively. In addition, *S. soda* affect *B. cinerea* mycelia growth by totally inhibition at 10 and 5 mg/ml, and by 75% at 2.5 mg/ml. *S. soda* has complete inhibition of spore germination at 10 mg/ml concentration for all fungal species tested. However, the number of colonies at 5 and 2.5 mg/ml concentration were the same, in comparison with negative control, but the diameter of each colony was reduced by 50%, that means the inhibition process is still working. *Salsola* species have been reported to have flavonoids and alkaloids, that might have the antimicrobial/antifungal activities (Tundis et al., 2009).

According to Table 8, we can see the minimum concentration value of ethanolic leaf extracts of the studied plant species that totally inhibit fungal growth using two different assay methods. We can conclude that the most sensitive fungus to the studied plant extracts was *B. cinerea*, which was completely inhibited at 5 and/or 2.5 mg/ml, followed by *C. sphaerospermum* and *A. alternata*. In addition, the least sensitive fungus was *F. oxysporum*, in which the concentration needed to totally inhibit its growth is 10 mg/ml or greater (Figure 16). Also, the Table 8 demonstrated that *L. axillare* has the highest effects on all fungal species with total inhibition of *B. cinerea* mycelia growth at the lowest concentration 2.5 mg/ml. *L. axillare* is a succulent plant that can grow in

hypersaline soil under the arid environment (Moreno, Terrones, Alonso, Juan, & Crespo, 2018). Some of *Limonium* species; such as *L. gmelinii*, *L. leptophyllum*, and *L. myrianthum* were shown antifungal, antibacterial, antimalarial and anti-leishmanial activities (Gadetskaya et al., 2016). They were reported to have flavonoids and phenolic compounds that might be a core for their activities (Gadetskaya et al., 2016).

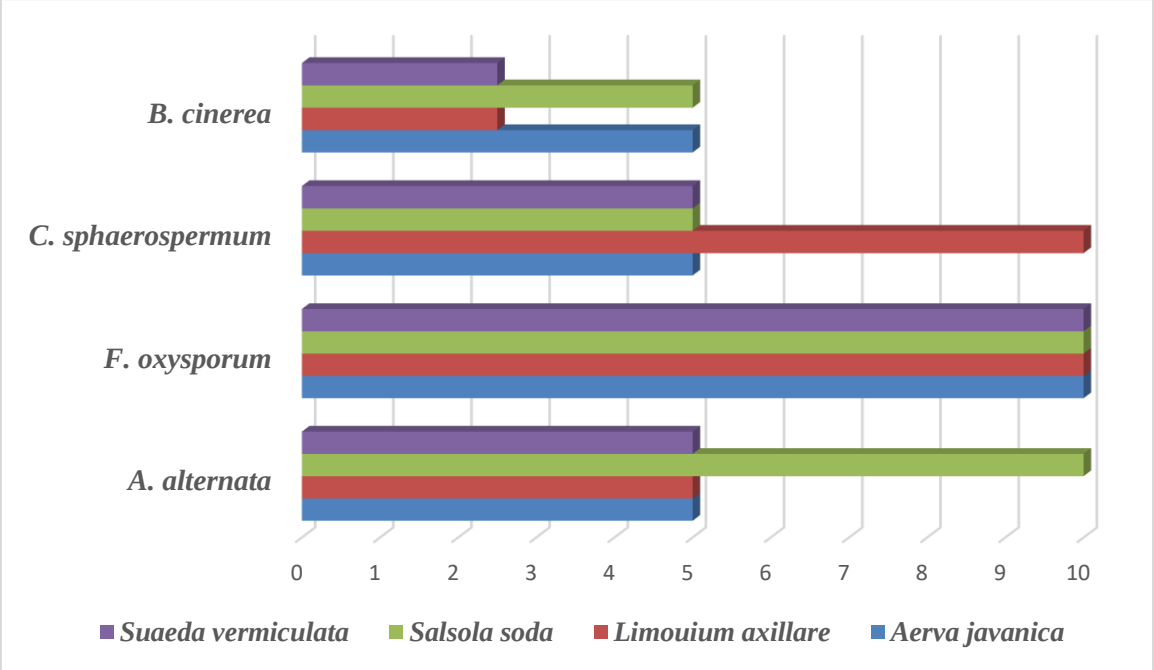

Figure 16. The lowest concentration values for ethanolic leaf extracts of the studied plant species that totally inhibit fungal growth using poisoned food methods.

5.2 Anticancer Activity Assay

Carcinogenesis is a result of inhibited apoptosis and activation of uncontrolled cell proliferation. Traditionally, medicinal plants have been used against numerous diseases because of their bioactive constituents, and some of these compounds have powerful anticancer activity. One of the aims of this study was to explore the anticancer activity of four unexplored Qatari medicinal plants *Aerva javanica, Limonium axillare, Salsola soda* and *Suaeda vermiculata*. We used breast cancer cell line, MCF-7, which was isolated from a primary tumor and is characterized by a wildtype p53. While three of the crude extracts isolated with water or methanol methods did not show any significant effects on MCF-7 cancer cell proliferation, the shooting part of *S. vermiculata* methanolic and water extracts showed a significant effect on cell proliferation at various increasing concentrations. Therapeutically, *S. vermiculata* contains polyphenols, including flavonoids, which exhibit cytotoxic activities in scavenging of free radicals (Cybulska et al., 2014). Thus, we conclude that the crude extract from *S. vermiculata* requires further comprehensive investigation, where normal epithelial breast cells should be used in comparison with additional breast cancer cells to provide additional data supporting its true anti-proliferative effect. In this case only, one should move to fractionation of the crude extract to identify the bioactive ingredient responsible for inhibiting BC cell proliferation and inducing cell death.

6. CONCLUSIONS

About 80% of the population depend on traditional herbal medicine as an alternative for primary health care (WHO, 2008). The active constituents of plant extracts, in terms of the natural source to treat many kinds of diseases, have been extensively studied. Research on medicinal plants includes a wide range of aspects related to human health among those: antioxidant and antimicrobial activities. From the current study, selected Qatari plants have shown effective results against many fungal species. The study demonstrated that the aqueous extract was not as effective as ethanolic extract on the studied species include *Suaeda vermiculata, Limonium axillare, Salsola soda* and *Aerva javanica,* which are native Qatari plant species. Ethanolic extract of all studied plants exerted strong antifungal activities against fungal species; *Alternaria alternata, Cladosporium sphaerospermum, Fusarium oxysporum, and Botrytis cinerea.* Two methods, the poisoned food and agar diffusion methods, were used to assess the antifungal activity. A concentration of 10 mg/ml of ethanolic extract of any of the studied plants was enough to inhibit the growth of all studied species of fungi completely. In other experiments, water and methanolic extracts of the above-mentioned plants were assessed against MCF-7 (breast) cell line. Results have shown a low cytotoxic activity from one plant species *S. vermiculata* at 400 µg/µl concentration.

This study highlights the importance of such research on Qatari flora and prompts the need for further research on different biological activities on different aspects related to human health. Widening of the research to include more plant species, and even more types of extracts from each plant are necessary to have more comprehensive knowledge

and provide baseline data for future research. Further research is also essential to isolate the bioactive compounds from the studied plants and to investigate which compound (s) is (are) the most effective.

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