

## Antimicrobial Activities of Some Plant Extracts against Some Fungal Pathogens

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**Abstract:** One of the major clinical problems is the resistance of fungal species towards most of the used antifungal agents. The search for new antifungal drug from plants helped to overcome this problem. This study evaluated the effects of some fruits extracts cited in the Holy Quran as antifungal agents. In this study, antifungal susceptibilities assessment of water and methanolic extracts of fruits extracts were done against *Aspergillus niger*, *Aspergillus parasiticus*, *Fusarium oxysporum*, and *Alternaria alternate* by well diffusion method. Microdilution susceptibility assay was used in order to determine the minimal inhibitory concentrations (MICs) of the plant extracts. Among the evaluated plants, methanolic extract was more effective than water extract. The inhibition zone of all extracts ranged from 7.9mm to 22.6 mm. The minimum inhibitory concentration (MIC) values of these crude extracts against tested fungal strains ranged between 3.12 to 625 mg/ml. All extracts were found to inhibit ( $p < 0.05$ ) the examined fungal strains. Garlic exhibits a significant antifungal activity against four fungal species. The antifungal activity of the selected plants may be due to presence of some chemical compounds such as terpenoids, flavonoids and phenols. In conclusion; these plants may have a potential use in pharmaceutical and preservative products. Further studies are needed to explore the novel antifungal bioactive molecules.

**Keywords:** Antifungal activity; Plant extract; MIC, Garlic, *Aspergillus*, *Alternaria*, *Fusarium*

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### I. Introduction

Cancers and microbial infections are still a major health problem; therefore research on new anticancer and antimicrobial agents ought to be continued. Natural products including fruits and medicinal plants continue to be an important resource to manage various diseases. Individuals suffering from malignancies are at high risk of contracting fungal infections. Even though developed treatments have improved the survival of cancer patients, increased fungal infection complications remain a leading cause of morbidity and mortality worldwide. About half of these patients are thought to have an infection as either the primary correlated cause of death. To prevent progression of the fungal infections, effective antifungal treatments are necessary (Chen *et al.*, 2016). Due to indiscriminate use of commercial antimicrobial drugs commonly used for the treatment of infectious disease, the resistances of the organisms have been increased. The issue of resistances and toxicity of some of these currently available antifungal agents compelled the researchers to search for antimicrobial substance from several sources including medicinal plants (Bansod *et al.*, 2008). During the last several decades, natural products with antimicrobial effect are investigated in order to eliminate the use of synthetic antibiotics which cause the resistance of microorganisms and can exhibit side effects to human health (Mihajilov-Krstev *et al.*, 2010). Plants appear as an interesting source for antifungal compounds, based on the knowledge that they develop their own defense against fungal pathogens. Many studies have been carried out to screen medicinal plants for their antifungal activity (Ahmad *et al.*, 2010).

Currently used antifungal agents include three major classes of drugs with different mechanisms of action: polyenes (disrupt fungal membranes), azoles (inhibit ergosterol biosynthesis), and echinocandins (inhibit synthesis of cell wall  $\beta$ -glucan). However, all current antifungal agents suffer from inefficiency, toxic side effects, drug-drug interactions and developing drug-resistance (Diallinas *et al.*, 2018). Humans and fungi share some of the same molecular processes; therefore, there is always the risk that what is toxic to the fungal cells may be toxic to the host cells (Arif *et al.*, 2009).

The economic crisis, high cost of industrialized medicines, inefficient public access to medical and pharmaceutical care, in addition to the side effects caused by synthetic drugs are some of the factors contributing to the central role of medicinal plants in health care. With the rise in-at risk patients, the number of invasive fungal infections has dramatically increased in both developed and developing countries. Potential pharmacological usages that take advantage of bioactive plant-derived compounds, antimicrobial, antifungal, anti-inflammatory, and antioxidant properties are being developed and many new ones explored. Traditional medicine has made use of many different plant extracts for treatment of fungal infections and some of these have been tested for in vitro antifungal activity.

In recent years, however, there is a growing interest in medicinal and aromatic plants (MAPs) and their antioxidant and oxidative stress modulation properties. This field of research looks particularly interesting for cancer therapeutics. A crucial aspect of the plant defense systems is the ability to distinguish between pathogens and beneficial interacting microorganisms (Farkaset *al.*, 2018). There are many edible and non-edible plants such as ginger, garlic, olive, pomegranate, grape and banana, which are cited in the Holy Quran, are used as remedies for various ailments such as cough, intestinal bleeding and diarrhea (Sheikh & Dixit, 2015). Therefore, plants mentioned in the Holy Quran have been attracted by the botanists, biochemists and pharmacognosist (Natural drug specialists) for research purposes. In the Holy Quran, about nineteen medicinal plants have been identified (Urbi *et al.*, 2014). Thus, the particular objectives of this study are to investigate antifungal activities of 15 species cited in The Holy Quran grown in Saudi Arabia.

## **II. Materials and Methods**

### ***Source of microorganisms***

Pathogenic fungi used in this study as test organisms were obtained from King Abdulaziz Hospital and Microbiology Lab., Faculty of Science, Jeddah, Saudi Arabia. The tested fungi were *Fusarium oxysporum*, *Aspergillus niger*, *A. parasiticus* and *Alternaria Alternata*. Fungal isolates were grown on PDA medium and preserved on slants of the same medium at 4°C until used.

### ***Collection of Plant Materials***

A total of 15 plant materials (8 fruits, 5 vegetables and 2 types of grains) were chosen from those cited in the Holy Quran. These food items were purchased from local markets in Makkah, Jeddah and Taif, Saudi Arabia, during summer 2017. They were identified at Biology Department, Faculty of Science, Jeddah, Saudi Arabia. The tested plants were ginger, onion, garlic, cucumber, fig, wheat, lentils, olive, gourd, date, banana, grape and pomegranate.

### ***Preparation of Plant Extracts***

#### ***i- Water Extraction***

Five gram of each ground plants was weighed and 50 ml of distilled water was added and shake. Then, the mixtures were heated and bring it to boil three times. After that, the mixtures were leaved until cooled and filtered through a filter paper (no. 1). Centrifugation and filtration process were repeated three times and stored in falcon tube (50ml) at -20°C until analyzed within two weeks.

#### ***ii- Methanol Extraction***

Five gram of each ground plants was weighed and 50 ml of methanol was added and leaved the whole night in dark place. Then, the mixtures were filtered through a filter paper (no. 1) into small beaker (50ml) and evaporated the methanol in a freeze-dryer. After that, 2 ml of Dimethyl sulfoxide was added to the mixtures to dissolve all the samples and scratch if necessary. Then, the samples were kept in Eppendorf tube (2 ml) at -4°C until analyzed within two weeks.

### ***Determination of Antifungal Activity***

All the tested fungi were grown on Potato Dextrose Agar which is a rich medium used for the cultivation of fungi for 4 days at 25°C. Each fungal was taken and shaken in the sterile distilled water and spore suspension was prepared, 10<sup>6</sup>CFU/ml. About 0.1 ml of the prepared fungal suspension was spread evenly onto the agar surface using sterilized cotton swab and leave for 2 hours. Wells (7 mm) were cut into the plates using sterile cork borer and 50µl of plant extracts was placed into each wells. All prepared plates were incubated at 28°C for 2-3 days. The diameter of the resulting inhibition zone (mm) around the wells was measured and the mean value of three readings was calculated and taken as a criterion for antimicrobial activity (Joshi *et al.*, 2009).

#### **Determination of Minimum Inhibitory Concentration (MIC)**

MIC was determined as the lowest concentration of extracts that inhibited visible growth of the test fungi after 2 days (Tradit *et al.*, 2011). Minimal inhibitory concentration was determined using Broth microdilution method. The tested fungal samples was used to evaluate the inhibitory activity of a plant extracts. Seeded broth corresponding to  $10^6$  spore/ml was added into 12 wells in a microtiter plate (50  $\mu$ l/well), then 50  $\mu$ l of the selected plant extracts was added to well number 1 and the mixture was mixed. A volume of 50  $\mu$ l of test material was serially diluted by two fold dilution method. The 96 well plates were incubated for 2-3 days and the MIC results were read visually. The experiment was carried out in triplicates (Joshi *et al.*, 2009).

#### **Statistical analysis**

Each test was performed in triplicate, and all data analysis was expressed as a mean  $\pm$  standard deviation. Significant differences between means were analyzed statistically using tow-way analysis of variance (ANOVA) according to Dunnett new multiple-range test through SPSS 17.0 (SPSS Inc., Chicago, USA) software package in Microsoft Windows 8.0 operating system. Differences were considered significant when  $p < 0.05$ . Statically: all extracts were found to significantly inhibit ( $p < 0.05$ ) the activity against fungal strains examined.

### **III. Results and Discussion**

Fungal infections constitute an emerging threat and a prevalent health problem due to increasing number of immunocompromised people and pharmacological or other treatments aiming at viral infections, cancer or allergies. Currently used antifungal agents suffer from inefficiency, toxic side effects and developing drug-resistance. Additionally, over the last two decades no new classes of antifungal agents have been approved, emphasizing the urgent need for developing a novel generation of antifungal agents (Diallinas *et al.*, 2018). Due to the development of adverse effects and microbial resistance to the chemically synthesized drugs, men turned to ethnopharmacognosy. They found literally thousands of phytochemicals from plants as safe and broadly effective alternatives with less adverse effect. Many beneficial biological activity such as anticancer, antimicrobial, antioxidant, antidiarrheal, analgesic and wound healing activity were reported. In many cases the people claim the good benefit of certain natural or herbal products (Tradit *et al.*, 2011).

Natural products from medicinal plants, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. Due to an increasing demand for chemical diversity in screening programs, seeking therapeutic drugs from natural products, interest particularly in edible plants has grown throughout the world (Tradit *et al.*, 2011).

The antimicrobial activities of plant extracts have formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies (Mahlo *et al.*, 2016). Medicinal plants played a crucial role in the search for components that are capable of neutralizing the multiple mechanisms of fungal resistance. The present paper reported the collection of medicinal plants mentioned in Holly Quran and used traditionally in KSA to treat some human pathogens.

The results in Table (1) showed the antifungal activity of water and methanol extracts of 15 tested plants against pathogenic fungi. Water is universal solvent, used as initial screening of the plant products with antimicrobial activity (Das *et al.*, 2010). For water extract, 3 out of the 15 plants water extract namely garlic, lentils, and wheat inhibited the growth of all tested fungi with zone of inhibition ranged from 8.3-22.6 mm and MICs from 3.12 to 50 mg/ml. The maximum inhibition zone diameter (antifungal effect) was 22.6 mm which was obtained for water extract of garlic against *Fusarium oxysporum* while nystatin, the control antifungal agent, gave 15 mm. Lower MIC of water extract was recorded for garlic with 3.12 mg/ml against both *Fusarium oxysporum* and *Aspergillus niger*. However, plant extracts from organic solvents have been found to give more consistent antimicrobial activity compared to water extract (Das *et al.*, 2010). For methanol extract, all plants exhibited antifungal activity pathogenic fungi with zone of inhibition ranged from 7.9-11.8 mm and MICs from 78.1 to 625 mg/ml. However, it is important to point out that 9 out of 15 plants extracts namely ginger, onion, garlic, cucumber, fig, wheat, lentils, gourd, and date extracts exhibited antimycotic against all tested fungi while the rest of plants exhibited the antifungal activity at least against two pathogenic fungi. Banana, grape and pomegranate have no inhibitory effect on *F. oxysporum*. Moreover, olive, pomegranate and red grape have no inhibitory effect against *A. parasiticus*. It was clear that all of the extracts have inhibitory activity against *A. niger* and *Alternaria alternata*.

Previous studies provide similar results for methanol as a better solvent for more consistent extraction of antimicrobial substances from medicinal plants as compared to other solvents such as water and ethanol (Ewais *et al.*, 2014). Plant products, particularly spices and extracts of various plant parts have been used extensively as natural antimicrobials and antioxidants. Garlic (*Allium sativum*) has been studied

not only for its benefits in controlling infection by bacteria, but also infection from other microbes including yeasts and fungi. Several studies have reported that garlic bulb extract can inhibit the growth of bacteria, fungi, and viruses in culture media and food systems (Yin & Cheng, 1998). Garlic extracts showed antimicrobial activity against all tested fungi with zone of inhibition ranged from 8.4 to 22.6 mm. The maximum zone (22.6 mm) of antifungal effect was observed with the water extract of garlic against *F. oxysporum* compared to nystatin (15 mm). Lower MIC of water extract was recorded for garlic with 3.12 mg/ml against *F. oxysporum* and *A. niger*. The antifungal activity of garlic bulb against *A. niger* was reported by Yin & Tsao (1999). The antifungal activity of essential oil of garlic extracts against *F. oxysporum* was reported by Benkeblia (2004). The antimicrobial activity of garlic is believed to be due to the effect of allicin (diallyl thiosulfinate), ajoene, and other sulfite compounds (Yin & Cheng, 1998). Allicin decomposes into various products, one of which, ajoene, resulting from the conjugation of three allicin molecules, has been reported to have antifungal activity (Slusarenko *et al.*, 2004). These sulfur compounds represent the majority of garlic chemical constituents, and it is strongly believed that they are the source of their pharmacological activity. Allicin is produced from the non-protein amino acid alliin (S-allylcysteine sulfoxide) upon tissue damage in a reaction that is catalyzed by the enzyme alliinase. Allicin reacts with low-molecular weight cellular thiols such as glutathione (GSH), shifting the GSH-based cellular redox-potential to a more oxidized state. Besides being redox active, allicin is also quite lipophilic indicating that allicin is membrane permeable and its antimicrobial activity is certainly facilitated by its ready entry into cells. At the same time, it has been shown that allicin is able to form transient pores in artificial and in bio-membranes, which perhaps accounts for its reported synergy with membrane-active antibiotics such as amphotericin B and polymixin B (Leontiev *et al.*, 2018). Furthermore, allicin is the antimicrobial agent which acts as partially inhibiting DNA and protein synthesis and also totally inhibiting RNA synthesis as a primary target. Organosulfur compounds and phenolic compounds have been reported to be involved in the garlic antimicrobial activity. Moreover, the antimicrobial potency of garlic is believed to be due to tannins, saponins, phenolic compounds, essential oils, and flavonoids (Karuppiyah & Rajaram, 2012). The activity of water extract of garlic might be due to the fact that allicin is well extracted in water (Sudarshan *et al.*, 2010). Onion (*Allium cepa*) exhibit antimicrobial effect against *B. subtilis*, *Salmonella* sp. and *E. coli* (Winston, 2008) and aflatoxin producing molds (Sharma *et al.*, 1979). Onion extract is a traditionally used in many cultures as antimicrobial agents. Nonetheless, there is still a dearth of scientific validation pertaining to the antibacterial and possible antibiotic potentiating activity of these plants.

The water extracts of onion have no antifungal activity. In disagreement with our results, Srinivasan *et al.*, (2001) have reported that the water extracts of onion exhibited activity against both filamentous and non-filamentous fungus. Shams-Ghahfarokhi *et al.*, (2006) have indicated that water extracts of onion might be promising in treatment of fungal-associated diseases from important pathogenic genera. Many factors could contribute to this variation, such as the plant variety, growing condition, maturity, season, geographic location, fertilizer used, soil type, storage conditions and amount of sunlight received. Other contributing factor for this difference may be also due to sample preparation and analytical procedures (Qusti *et al.*, 2010). The fact that allicin is well extracted in water (Sudarshan *et al.*, 2010) and garlic contains nearly three times as much sulfur-containing compound as onions (Benkeblia, 2004) could be the explanation for the stronger activity of garlic in our study. However, the methanol extract of onion has antifungal activity against all tested fungi. The antimycotic activity was ranged from 9.3 mm to 10.5 mm with MICs were 156.2-312.5 mg/ml. Onion extracts exert potent antimycotic properties (Yin & Tsao, 1999). Chintkuntla, (2015) had reported that onion extracts possess an antifungal activity. The essential oil of red onion extracts showed antifungal activity toward *F. oxysporum* and *A. niger* (Benkeblia, 2004). A novel antifungal compound, fistulosin (octadecyl 3-hydroxyindole) was isolated from *A. fistulosum* which showed high activity towards *Fusarium oxysporum* primarily inhibiting protein synthesis. The alk(en)yl cysteine sulphoxides (ACSOs), proteins, saponins and phenolic compounds may contribute to this activity (Griffiths *et al.*, 2002). Concerning the antimicrobial activity, garlic was more active than onion, which agrees with the literature. The same tested plant extracts has excellent antibacterial activities (Qusti *et al.*, 2018) and antifungal activity of both extracts (Krstin *et al.*, 2018).

Ginger (*Zingiber officinale*) is a medicinal plant mentioned before. In the present study, the methanol extracts of ginger has antifungal activities against all tested fungi. The antimycotic activity was ranged from 9.9 mm to 11.8 mm with MIC was 78.1 mg/ml for *F. oxysporum* and *A. alternata* and 156.2 mg/ml for *Aspergillus* species. The volatile oil components in ginger consist mainly of sesquiterpene hydrocarbons, predominantly zingiberene (35%), curcumene (18%) and farnesene (10%), with lesser amounts of bisabolene and  $\beta$ -sesquiphellandrene. A smaller percentage of at least 40 different monoterpenoid hydrocarbons are present with 1,8-cineole, linalool, borneol, neral, and geraniol being the most abundant. Many of these volatile oil constituents contribute to the distinct aroma and taste of ginger. Furthermore, ginger contains biologically active constituents including the non-volatile pungent principles, such as the gingerols, shogaols, paradols and zingerone that produce a "hot" sensation in the mouth (Shukla & Singh, 2007). Singh *et al.*, (2008) have reported the antifungal activity of ginger oil and oleoresin against *A. niger*. Ginger essential oil and oleoresins contain

considerable amounts of phenolic compounds (eugenol, shogaols, zingerone, gingerdiols, gingerols, etc.), which might be responsible for the observed antimicrobial potency. Essential oils contain two main components, terpenes and aromatic compounds were investigated for their inhibitory effects on the growth of many bacteria and fungi (Tu *et al.*, 2018). Antifungal activity was strongly associated with monoterpenic phenols (Isman, 2000). Ginger effectively inhibited *in vitro* mycelial growth and spore germination of *Pestalotiopsis microspora*, and exerted antifungal activity *via* membrane-targeted mechanism with alteration of membrane permeability, collapse of membrane integrity, and membrane lipid peroxidation. The ginger treatments destroyed the morphology of the mycelia increasing the leakage of intercellular electrolytes, proteins, sugars, and nuclein of *P. microspora*, leading to lethal effects on the pathogen (Chen *et al.*, 2018). Ficker *et al.*, (2003) have found that ginger had pronounced antifungal activity against a wide variety of fungi and it is generally regarded as safe for human consumption. According to Latif *et al.*, (2006), plant extracts prepared from garlic, ginger and onion bulb were capable to control *Alternaria*, *Aspergillus*, and *Fusarium* (Bokhari, 2012).

Starting with the base level of the pyramid we have breads and cereals that have been advised by doctors and nutrition therapists to be consumed in a larger proportion of servings than other food groups. For thousands of years, cereals have formed the staple diet essential for human life. Wheat and lentils are excellent source of protein and also rich in vitamins and minerals. According to Qusti *et al.*, (2010), they showed both antioxidant activity and phenolic content. The major components with antioxidant activity in wheat belong to the group of phenolic acids, which are mostly found in the bran, suggesting the use of wheat grain as whole instead of refined (Varieties *et al.*, 2017). They contained a diverse array of bioactive compounds that may contribute to their antifungal activity such as flavonoids, phytic acid, phenolic acids, and tocopherols. The water extracts of wheat and lentil showed antimicrobial activity against all tested pathogenic fungi. The activity of wheat was ranged from 9.1 mm to 10.7mm while the activity of aqueous extract of lentil was ranged from 8.3mm to 10.4mm with minimal inhibitory MICs values between 25 and 50 mg/ml for both. Moreover, the methanol extracts of lentil and wheat exhibited activity against all tested fungi with MICs values 312.5 mg/ml. Previous studies provide that they both possess antifungal proteins which is endowed with ribonuclease activity and able to enter inside fungal cells leaving the cell wall intact. Once entered in the cytoplasm of host cells the antifungal activity of the protein can be exerted on the basis of its ribonuclease activity on endogenous RNA. pathogenesis-related (PR) proteins defined as proteins encoded by the host plant but induced specifically in pathological situations (Bertini *et al.*, 2009). Meloen *et al.*, (1997) have demonstrated that the PR-1 proteins have antifungal activity at the micromolar level against a number of plant pathogenic fungi which have been found in wheat. According to Terras *et al.*, (1993) the wheat thionin inhibit of fungal growth. Wang and Ng (2007) have reported that the lentil antifungal peptide is a lectin-like peptide exhibited antifungal activity against *Fusarium oxysporum*. Lectins are carbohydrate-binding proteins and their biological properties include cell-cell interactions. The polysaccharide chitin is constituent of fungi cell wall and chitin-binding lectins showed antifungal activity; impairment of synthesis and/or deposition of chitin in cell wall may be the reasons of antifungal action (Paiva *et al.*, 2010).

The methanol extracts of Cucumber (*Cucumis sativus*) exerted antimycotic effect ranged from 9 mm to 9.7 mm with MIC was 312.5 mg/ml against tested pathogens. Cucumber possessed phytoconstituents such as glycoside, steroid, flavonoid, saponin and tannin, and carbohydrates (Rajasree *et al.*, 2016). The toxic effect of saponins to fungi is related to the ability of these compounds to form a complex with membrane sterols which lead to pore formation (Osbourn, 1996). The cucumber Fruit contains rutin which is a flavonol and the seeds contain glucosides including cucurbitaside (Sheikh and Dixit, 2015). Chitinases have been reported cucumber a potent antifungal activity against human and plant pathogens such as *F. oxysporum* (Meloen *et al.*, 1997).

Figs (*Ficus carica*) can trace their history back to the earliest of times with mentions in the Bible and other ancient writings. They are thought to have been first cultivated in Egypt. They spread to ancient Crete and then subsequently, around the 9th century BC, to ancient Greece, where they became a staple foodstuff in the traditional diet. Figs were held in such esteem by the Greeks that they created laws forbidding the export of the best quality figs. Figs were also revered in ancient Rome where they were thought of as a sacred fruit (Farhangi *et al.*, 2014).

Olive (*Olea europe*) and fig (*Ficus carica*) are amongst those herbal medicines that are frequently studied and documented to have beneficial anti-inflammatory, immune modulator, antimicrobial, anticancer, chemopreventive, analgesic and anti-oxidant effects. They mainly owe their biologic properties to oleic acid and phenolic components. Furthermore, lupeol, a dietary triterpene found in olive and fig fruit, has shown anti-inflammatory, anti-arthritic, anti-mutagenic and anti-malarial activity. In addition, based on ethnopharmacological documents and folkloric believes, olive and fig have been used in inflammatory disorders such as inflammatory swellings and hard swellings (Bahadori *et al.*, 2016).

The methanol extract of fig (*Ficus carica*) fruit possessed antifungal effect ranged from 8.8 mm to 9.6 mm with MIC was 312.5 mg/ml against tested pathogens. The results obtained in our screen are in agreement with published results for Shirata and Takabashi (1982) which reported that the extract of fig possessed

antifungal activity (Mousa *et al.*, 1994). The methanolic extracts showed a strong antimicrobial effect. This effect might be attributed to the high content of flavonoids and tannin (Debibet *et al.*, 2014). Fig contains many antioxidants, and it is a good source of flavonoids and polyphenols and some bioactive compounds such as arabinose,  $\beta$ -carotenes, and glycosides (Joseph & Raj, 2011). Phytochemical studies on the leaves and fruits of the fig have shown that it is rich in phenolics, organic acids, and volatile compounds. The further phytochemical investigation revealed different composition in the coumarin, fatty acid, and flavonoid content (Rameshrad *et al.*, 2015). Coumarins have been found to stimulate macrophages, which could have an indirect negative effect on infections (Cowan, 1999).

The methanol extracts of olive (*Olea europaea*) were antifungal against *A. niger*, *F. oxysporum* and *A. alternata*. The inhibition zone was ranged from 9.1 mm to 10.4 mm with MIC was 312.5 mg/ml for *A. niger* and *F. oxysporum*, and 156.2 mg/ml for *A. alternata*. Upadhyay (2014) has reported the inhibitory effect of olive oil on *A. niger*. Some workers have been reported that the antimicrobial properties of phenolic compounds in olive products refer to compounds obtained from olive fruit, particularly hydroxytyrosol and oleuropein (Pereira *et al.*, 2007). Olive fruits extracts has been reported for their antimicrobial and bacteriostatic activity since 1970. Mild antifungal activity has been also described for olive leaf extracts and the major olive phenolic compound oleuropein (Diallinas *et al.*, 2018). Phytochemical research had led to the isolation of flavonoids, secoiridoids, iridoids, flavanones, biophenols, triterpenes, benzoic acid derivatives, isochromans, and other classes of secondary metabolites from olive. The plant materials and isolated components have shown a wide spectrum of *in vitro* and *in vivo* pharmacological activities like antidiabetic, anticonvulsant, antioxidant, anti-inflammatory, immunomodulatory, analgesic, antimicrobial, antiviral, antihypertensive, anticancer, antihyperglycemic, antinociceptive, gastroprotective and wound healing activities (Hashmi *et al.*, 2015). A series of long-chain  $\alpha$ ,  $\beta$  unsaturated aldehydes from olive fruit and its oil flavor for their antimicrobial activities and found them active against broad spectrum of microbes (Hashmi *et al.*, 2015). Zori *et al.*, (2016) suggest that oleuropein, a complex phenol present in large quantities in olive tree products, against targets virulence factors essential for establishment of the fungal infection. They noticed that oleuropein modulates morphogenetic conversion and inhibits filamentation of *Candida albicans*. It was also demonstrated that the tested compound inhibits the activity of SAPs, cellular enzymes secreted by *C. albicans*, which are reported to be related to the pathogenicity of the fungi. Furthermore, they detected that oleuropein, a complex phenol present in large quantities in olive tree products, causes a reduction in total sterol content in the membrane of *C. albicans* cells, which might be involved in the mechanism of its antifungal activity (Zori *et al.*, 2016). Hydroxytyrosol is one of the main phenolic compounds in olives (Tripoli *et al.*, 2005). It is a phenyl ethanoid, a type of phenolic phytochemical with antioxidant properties *in vitro*. The olives, leaves and olive pulp contain large amounts of Hydroxytyrosol. Diallinas *et al.*, (2018) conclude that the direct target of olive and the synthesized Hydroxytyrosol analogs is disruption of the fungal plasma membrane integrity and function. The methanol extracts of Gourd (*Cucurbita pepo*) were antifungal against tested fungi. The antimycotic activity was 9.4 mm for *A. niger* and 9.1 mm for *A. parasiticus*, and *F. oxysporum* with MIC was 312.5 mg/ml. An important study showed that the gourd possessed antifungal activity against *F. oxysporum*. Pharmacologically, it is used for different activities like anti-hypercholesterolemia, anti-hypertensive, antiparasitic, antidiabetic, anticarcinogenic, antibacterial, anti-inflammation, antitumor and antioxidant. Different categories of phyto-constituents contain in gourd such as linoleic acids, oleic acid, alkaloids, flavonoids and palmitic which may be responsible for its medicinal properties (Adnan *et al.*, 2017). It exerted antifungal activity against *Fusarium oxysporum* (Tomar *et al.*, 2014). Phytochemicals such as flavonoids, polyphenolics, terpenoides, saponins, proteins and carbohydrates are also present. Terpenoides have membrane disruption and inhibitory effect against fungi (Rajasree *et al.*, 2016). The date-palm (*Phoenix dactylifera*) has been the most frequently mentioned. The Date palm is a well-known fruits all over the world. This fruit was the main source of foods in ancient time and currently, many products of food have been prepared from dates (Hossain *et al.*, 2016). The methanol extracts of date were antifungal against tested fungi. The antimycotic activity was ranged from 8.7 mm to 9.5 mm with MIC were 625 mg/ml for tested fungi. Bokhari, (2012) have reported that the methanol extracts of leaves and pits of date inhibited the growth of *F. oxysporum*, *A. alternata*. The Fruit of dates were found to be a high source of antioxidants, anthocyanins, carotenoids and phenolics. Furthermore, it contains sterols, procyanidins, and flavonoids. The most effective antioxidants in this respect appear to be the flavonoids and phenolics (Ateeq *et al.*, 2013; Alam, 2014). The positive effect might be due to the selective or synergistic action of various chemicals present in date palm (Bokhari, 2012).

The methanol extracts of Banana (*Musa Cavendish*) were antimycotic against *A. niger*, *A. parasiticus* and *A. alternata*. The antifungal activity was ranged from 8.4 to 9.3 mm with MIC were 312.5 mg/ml for *A. niger*, *A. parasiticus*, and *A. alternata* and have no effect on *F. oxysporum*. Yasmin and Saleem, (2014) have been reported the effect of crude banana extract which inhibited the growth of *F. oxysporum* and *A. niger*. Vincent *et al.*, (2007) have reported that Thaumatococin-like antifungal protein from Cavendish exerts antifungal activity against *F. oxysporum* and *A. niger* (Yasmin & Saleem, 2014). Banana pulp contains various phenolic

compounds, such as gallic acid, catechin, epicatechin, condensed tannins and anthocyanins. Cowan, (1999) have reported that the catechins, the most reduced form of the C3 unit in flavonoid compounds, inhibited in vitro microorganisms. Banana contains high amounts of total phenolic compounds and flavonols (Singh et al., 2016). The flavonoids activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls (Cowan, 1999). The mechanisms thought to be responsible for phenolic toxicity to microorganisms include enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl groups or through more nonspecific interactions with the proteins (Cowan, 1999). The flavonoids activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls (Cowan, 1999).

Regular consumption of fruits and vegetables has been linked to reduced risk of developing chronic diseases. Compounds from grape (*Vitis vinifera*) have a range of health benefits which include antioxidant, anticancer, antibacterial and antidiabetic activities as well as cardioprotective, hepatoprotective and neuroprotective effects (Nassiri-Asl & Hosseinzadeh, 2016). In the present study, the inhibitory activity of methanol extracts of grape was ranged from 7.9mm to 10.7mm with MIC was 625 mg/ml. White grape was antifungal against all tested pathogenic fungi except *F. oxysporum* while red grape exhibited activity against *A. niger* and *A. alternata*. De Beer & Vivier, (2008) have reported that *Vitis vinifera* antimicrobial peptide 1, Vv-AMP1, was isolated from *Vitis vinifera* and shown to encode to a plant defense. Recombinant Vv-AMP1 displayed non-morphogenic antifungal activity against a broad spectrum of fungi, probably altering the membrane permeability of the fungal pathogens. The expression of this peptide is highly regulated in *Vitis vinifera*, hinting at an important defense role during berry-ripening. Salzman et al., (1998) have demonstrated that the accumulation of hexoses in grape is accompanied by an increase in a suite of protein that is homologous to proteins known to be antifungal determinants. These proteins have been identified as a thaumatin-like protein. Furthermore, It has been proposed that alkaloids and terpenoids may provide astringent antifungal properties (Kahrizi et al., 2012). The mechanism of action of terpenes is not fully understood but is speculated to involve membrane disruption by the lipophilic compounds (Cowan, 1999). Moreover, the resveratrol (3,4',5-trihydroxystilbene), which possess antioxidant, anti-inflammatory and anticancer properties, is a naturally occurring polyphenol stilbene which possess antifungal activity that found largely in grapes (Urbi et al., 2014, Kahrizi et al., 2012). Pomegranates contain a plentiful supply of potassium as well as such minerals as phosphorus, calcium, iron, and sodium, and vitamins A, B1, B2, B3, and C. Acting together with sodium, potassium regulates the body's water equilibrium and ensures that the heart beats normally. Pomegranates are used medicinally for diarrhea, earache, bad vision, fevers, teeth and gum disorders. Apart from its nutritious value, the pomegranate also has medical benefits. Greek, Egyptian, Arabic and Indian medicine, is a healing system based on scientific data and holistic principles, while homeopathy is a system of medicine that is based on natural laws applied therapeutically some 200 years ago (Farhangi et al., 2014).

The methanol extracts of Pomegranate (*Punica granatum*) antifungal against *A. alternata* and *A. niger* and the antimycotic activity was 8.3mm for *A. niger* and 8.1 mm for *A. alternata* with MIC was 625 mg/ml for both. Duman et al., (2009) have demonstrated that the pomegranate fruit have antibacterial and antifungal potential and their results indicated that pomegranate cultivars high in acid and rich in phenolics and anthocyanins have higher antibacterial and antifungal activity and this is closely correlated with pomegranate antioxidant capacity. The major class of pomegranate phytochemicals is the polyphenols include flavonoids (flavonols, flavanols and anthocyanins), condensed tannins (proanthocyanidins) and hydrolysable tannins (ellagitannins and gallotannins) (Dahham et al., 2010). The antimicrobial action of tannins may be related to their ability to inactivate microbial adhesions, enzymes, cell envelope transport proteins, etc. They also complex with polysaccharide (Cowan, 1999).

#### IV. Conclusion

Due to the fact that plant extracts usually occur as a combination of various type of bioactive compounds or phytochemicals, the reports of many studies about 15 plants studied here can be seen as a potential source of useful drugs since its extracts have shown to have an antioxidant, antimicrobial, and some other therapeutic properties which can contain preventative effect in many serious diseases. Further studies are required for finding the bioactive molecules responsible for the antifungal activity and determine their toxicity, side effects and pharmaco-kinetic properties.

**Table 1:** The Antifungal Activities (diameter of inhibition zone (mm)) of water and methanol extract of 15 tested plants against pathogenic fungi

Scientific Name	Common Name	Pathogenic Isolates								
		<i>Aspergillus niger</i>		<i>Aspergillus parasiticus</i>		<i>Alternaria alternata</i>		<i>Fusarium Oxysporum</i>		
		Water Extract	Methanol Extract	Water Extract	Methanol Extract	Water Extract	Methanol Extract	Water Extract	Methanol Extract	
1	<i>Allium sativum</i>	Garlic	22.5±0.3	8.4±0.1	11.7±0.1	9.5±0.2	11.7±0.3	11.2±0.2	22.6±0.1	10.4±0.1
2	<i>Zingiber officinale</i>	Ginger	ND	9.9±0.1	ND	10.6±0.1	ND	11.8±0.2	ND	11.1±0.1
3	<i>Triticum durum</i>	Wheat	9.1±0.1	8.8±0.1	9.1±0.9	9.6±0.1	10.8±0.1	9±0.3	10.7±0.4	9.7±0.1
4	<i>Lens culinaris</i>	Lentils	8.3±0.1	9±0.4	9.1±0.3	9.2±0.1	9.7±0.3	9.6±0.6	10.4±0.1	9.7±0.3
5	<i>Allium Pepo</i>	Onion	ND	9.3±0.4	ND	9.8±0.1	ND	10.3±0.1	ND	10.5±0.2
6	<i>Olea europaea</i>	Green Olive	ND	9.8±0.3	ND	ND	ND	10.2±0.6	ND	9.9±0.6
7	<i>Olea europaea</i>	Black Olive	ND	9.1±0.1	ND	ND	ND	9.3±0.4	ND	9.8±0.4
8	<i>Cucumis sativus</i>	Cucumber	ND	9±0.3	ND	9.7±0.1	ND	9±0.3	ND	9.5±0.1
9	<i>Ficus sycomorus</i>	Fig	ND	9±0.1	ND	9.2±0.1	ND	9.6±0.6	ND	8.9±0.2
10	<i>Cucurbita pepo</i>	Gourd	ND	9.4±0.2	ND	9.1±0.1	ND	8.9±0.2	ND	9.1±0.3
11	<i>Musa Cavendish</i>	Banana	ND	8.9±0.1	ND	9.3±0.3	ND	8.4±0.2	ND	ND
12	<i>Phoenix dactylifera</i>	Date	ND	8.7±0.4	ND	9.5±0.3	ND	9.4±0.3	ND	8.8±0.1
13	<i>Vitis vinifera</i>	White Grape	ND	8.4±0.3	ND	10.7±0.3	ND	10.6±0.4	ND	ND
14	<i>Vitis vinifera</i>	Red Grape	ND	7.9±0.3	ND	ND	ND	8.3±0.5	ND	ND
15	<i>Punica granatum</i>	pomegranate	ND	8.3±0.4	ND	ND	ND	8.1±0.1	ND	ND
<b>Nystatin (control)</b>			22 ± 0.00		24± 0.00		34± 0.00		15± 0.00	

ND:Not detected

**Table (2):** MIC expressed in mg/ml of methanol and water extract of 15 plants against some pathogenic fungi

Scientific Name	Common Name	Pathogenic isolates								
		<i>Aspergillus niger</i>		<i>Aspergillus parasiticus</i>		<i>Alternaria alternata</i>		<i>Fusarium oxysporum</i>		
		Water Extract	Methanol Extract	Water Extract	Methanol Extract	Water Extract	Methanol Extract	Water Extract	Methanol Extract	
1	<i>Allium sativum</i>	Garlic	3.12	312.5	12.5	156.2	6.25	156.2	3.12	156.2
2	<i>Zingiber officinale</i>	Ginger	-	156.2	-	156.2	-	78.1	-	78.1
3	<i>Triticum durum</i>	Wheat	25	312.5	50	312.5	25	312.5	25	312.5
4	<i>Lens culinaris</i>	Lentils	25	312.5	50	312.5	25	312.5	25	312.5
5	<i>Allium Pepo</i>	Onion	-	156.2	-	312.5	-	156.2	-	156.2
6	<i>Olea europaea</i>	Green Olive	-	312.5	-	-	-	156.2	-	312.5
7	<i>Olea europaea</i>	Black Olive	-	312.5	-	-	-	156.2	-	312.5
8	<i>Cucumis sativus</i>	Cucumber	-	312.5	-	312.5	-	156.2	-	312.5
9	<i>Ficus sycomorus</i>	Fig	-	312.5	-	312.5	-	312.5	-	312.5
10	<i>Cucurbita pepo</i>	Gourd	-	312.5	-	625	-	625	-	312.5
11	<i>Musa Cavendish</i>	Banana	-	625	-	312.5	-	625	-	-
12	<i>Phoenix dactylifera</i>	Date	-	625	-	625	-	625	-	625
13	<i>Vitis vinifera</i>	White Grape	-	625	-	625	-	625	-	-
14	<i>Vitis vinifera</i>	Red Grape	-	625	-	-	-	625	-	-
15	<i>Punica granatum</i>	pomegranate	-	625	-	-	-	625	-	-

- : not tested



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