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Original Article

Effect of Oleo-Gum Resin of Ferula Assafoetida on Growth of Some Food and Crop Contaminating Microbes

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ABSTRACT

Objective: The aim of this study was to determine the Minimum inhibitory concentration (MIC) and minimum fungicidal or bactericidal concentration (MFC, MBC) of oleo-gum resin of *Ferula assafoetida* on the growth of food spoilage microbes of bacteria, yeasts and fungal and investigation of the most sensitive of them to oleo-gum resin of these plant. **Methods:** In this study oleo-gum resin of F.assafoetida were dissolved in sterile distilled water and then its antimicrobial effects was studied on the growth of seven microbial species including Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Saccharomyces cereviciae, Candida albicans, Aspergillus flavus and Aspergillus parasiticus using micro-dilution method. **Results:** Results of these research showed that MIC and MBC values of oleo-gum resin on *B.subtilis* and *E.coli* were 1562.5 and >100000 µg/ml respectively, which were more than MIC and MBC values of *S.aureus.* Among tested yeasts, *C.albicans* showed more resistance than the *S.cereviciae.* Also MIC value of *A.parasiticus* (390.6 µg/ml) was less than *A.flavus* (781.3 µg/ml). Results of the present study indicates that oleo-gum resin of *F.assafoetida* has significant ($P<0.05$) antimicrobial activity, which strengthens its potential use as essential antimicrobial source in the near future.

1.INTRODUCTION

There is growing concern about food safety and especially about inputs of pathogenic microorganisms with possible implications for human health and the environment [Duniere et al., 2011]. Microbial activity is a primary mode of deterioration of many foods and is often responsible for the loss of quality and safety. Concern over pathogenic and spoilage microorganisms in foods is increasing due to the increase in outbreaks of foodborne disease [Ozturk and Ercisli., 2007]. It has been estimated that as many as 30% of people in the industrialized countries suffer from food borne diseases each year caused by microbes [Burt, 2004]. Food additives have been used for centuries in the food processing practices for several purposes including the prevention of microbial growth and increase in the food shelf lives [Ditschun and Winter, 2000]. Due to the excessive use of food preservatives which some of them are doubtful carcinogenic and teratogenic and also increasing consumer demand for natural foods with a long shelf life and without chemical preservatives, food producers tend to replace chemical preservatives with natural forms such as oils and herbal extracts as antibacterial additives [Bluma et al., 2008 and Mahzooni-Kachapi et al., 2011].

In the recent years, efforts have been devoted to finding new antimicrobial materials from natural resources for food preservation [Reddy et al., 2010]. Reports indicated that many extracts and essential oils of edible plants had properties to prevent against a wide range of fungal

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contamination of foods [Bluma et al., 2008 and Reddy et al., 2010 and Gowda et al., 2004 and Mohammadi et al., 2009 and Vaishnavi et al., 2007].

Assafoetida is an oleo-gum resin obtained from the exudates of the roots of the Iranian endemic medicinal plant, *Ferula assafoetida*. *F.assafoetida* grows wildly in the central and southern mountains of Iran. The oleo-gum resin assafoetida is called "Anghouzeh", "Khorakoma" and "Anguzakoma" in Iran. The plant, which belongs to the Apiaceae family, is an herbaceous perennial with an unpleasant odor that grows to about 2m in height. The oleo-gum-resin is often obtained by incision of the roots or removal of the stems. Hardened exudates (oleo-gum resin) are then collected and packed for export [Iranshahy et al., 2011]. Assafoetida has been used as a spice and a folk phytomedicine for centuries. It is used as a flavoring spice in a variety of foods, particularly in India [Iranshahy et al., 2011]. In Nepal assafoetida is considered to be a sedative, carminative, antispasmodic and a diuretic in its properties. It is also believed it has aphrodisiac properties which increases the sexual appetite. Indeed, assafoetida is consumed regularly in the daily Nepali diet [Duniere et al., 2011]. It is traditionally used for the treatment of different diseases, such as asthma, epilepsy, stomachache, flatulence, intestinal parasites, weak digestion and influenza [Iranshahy et al., 2011 and Zargari, 1995]. It is popular household remedies and its components are used for many prescriptions in traditional healing [Abd el-razek et al., 2001].

Recent pharmacological and biological studies have also shown several activities, such as antioxidant [Dehpour et al., 2009], antiviral [Lee et al., 2009 and Rollinger et al., 2008], antifungal [Gowda et al., 2004 and Angelini et al., 2009 and Kamble and Patil, 2008 and Rani et al, 2009 and Sitara et al., 2008], antibacterial [Vaishnavi et al., 2007 and Garg et al, 1980 and Kunwar et al, 2010 and Martinez et al, 1996 and Rahman et al., 2008 and Sasikumar et al., 2007], cancer chemopreventive [Iranshahi et al., 2008 and Iranshahi et al., 2009b and Mallikarjuna et al., 2003 and Saleem et al., 2001], anti-diabetic [Abu-Zaiton, 2010], insecticide [Moharramipour and Nazemi, 2008], antiparasite [Barati et al., 2010 and Sarkari et al., 2009] from this oleo-gum resin. Assafoetida consists of three main fractions, including resin (40–64%, which contains ferulic acid and its esters, coumarins, sesquiterpene coumarins and other terpenoids.), gum (25%, including glucose, galactose, l-arabinose, rhamnose, glucuronic acid, polysaccharides and glycoproteins) and essential oil (10–17% , including sulfur-containing compounds, monoterpenes and other volatile terpenoids) [Iranshahy et al., 2011 and Gholamnezhad et al., 2011]. The aims of the present study were to evaluate the potential antimicrobial activities of oleo-gum resin of *F.assafoetida* collected from Iran on the growth of some bacteria, yeasts and fungi.

2. MATERIALS AND METHODS

2.1. Plant and extraction

Oleo-gum resin of *F.assafoetida* was purchased in 2011 from Tabas, in Iran. The oleo-gum resin was confirmed by Medicinal Plants Institute, Ferdowsi University, Mashhad, Iran. The solid oleo-gum resin (20 g) was dissolved in distilled water (100 ml), warmed (mild warming: 40- 50°C) for 5-10 minutes in bath water (Dena, Iran) for complete dissolution, and filtered to eliminate waste and impurities [Gholamnezhad et al., 2011]. Concentrations and doses of the aqueous extract were expressed as total amount of the oleo-gum resin used in preparing the extract. The concentration of assafoetida in the stock solution was 200000µg/ml.

2.2. Source of organisms and preparation of suspension

A total of three bacteria (*Bacillus subtilis* ATCC 6633, *Escherichia coli 0157* NTCC 12900 and *Staphylococcus aureus* ATCC 6538*)*, two yeasts (*Saccharomyces cereviciae* 5052 PTCC and *Candida albicans* ATCC 10231), two fungi species (*Aspergillus flavus* PTCC 5006 and *Aspergillus parasiticus* PTCC 5018), obtained from Persian Type Culture Collection (PTCC), Iran, were used in the present study. To prepare microbial suspension, bacterial species were cultivated on nutrient agar (Merck, Germany) slant at 37^{o}C for 24 h while yeasts and fungal species were cultivated on PDA (Merck, Germany) slants and incubated at 25^{o}C for 48 h. Finally, suspensions were adjusted to 0.5 McFarland standard turbidity [Ozturk and Ercisli., 2007 and NCCLS, 2006 and NCCLS, 2002 and Kursat and Erecevit, 2009]. The yeasts and fungal suspensions were adjusted to make a conidial or spores concentrations of 10^6 cell or spore/ml via counting with a hemacytometer [Aoudou et al, 2012 and Khosravi et al., 2011 and Naeini et al., 2011 and Sanchez et al., 2005]. Bacterial suspensions were standardized to concentrations of 1.5×10^8 CFU/ml [Ozturk and Ercisli., 2007 and NCCLS, 2006].

2.3. Minimum Inhibitory Concentration (MIC) Test

Solution of oleo-gum resin of *F.assafetida* was first diluted to the highest concentration (200000µg/ml) and later, in twofold serial dilutions, made in a concentration range between 100000 µg/ml to 195.31 µg/ml. MIC values of solution of oleo-gum resin of *F.assafoetida* against microbial strains were determined based on a microwell dilution method. Ninety five µl of Mullerhinton broth (Merck, Germany) was dispended into each of 96 wells. 100 µl of stock solution of oleo-gum resin of *F.assafoetida* (200000µg/ml) was added into the first row of the wells. Then 100 µl from their serial dilutions was transferred into other consecutive wells with the exception of the well number 11 as positive control, and 5 µl of the microbial suspension added to each well with

exception of well number 12 as negative control. Contents of wells were mixed on a plate shaker at 300 rpm for 20 s and then incubated at 25°c for 48 h for yeasts and fungi and 37°c for 24 h for bacterial strains. Microbial growth was determined by detecting the absorbance at 630 nm using the ELX808 Elisa reader (Biotek Instrument Inc, USA). The MIC of solution was taken as the lowest concentration that showed no growth [Ozturk and Ercisli., 2007 and Mohammadi et al., 2009 and NCCLS, 2006 and NCCLS, 2004 and Gulluce et al., 2007].

2.4. Minimum Fungicidal or Bactericidal Concentration (MFC or MBC) Test

The minimum, fungicidal and bactericidal, concentrations (MFC and MBC) were determined with sub-culturing 10µl aliquot from all MIC wells showing no visible growth on the mullerhinton agar plates [Khosravi et al., 2011].

2.5. Statistical Analyses

All data obtained from the trial were analyzed as a completely randomized design using the procedure of the general linear model of SPSS 19 software (SPSS Inc., Chicago, IL, USA). The mean values were compared using Duncan's new multiple range test at 5% probability level of significance.

3. RESULTS AND DISCUSSION

4.1. Evaluation effect of oleo-gum resin of F.assafoetida on the growth of microbial species

Antimicrobial activity of oleo-gum resin of *F.assafoetida* was determined via the microwell dilution method at 10 concentrations against seven microorganisms that mainly contaminate foods. The results of in vitro antimicrobial activity assay showed that the oleo-gum resin of *F.assafoetida* possessed broad antimicrobial activity against the microorganisms tested. The antimicrobial effects of the solution of oleogum resin against seven microorganisms are shown in Table 1.

Table 1.

Minimum inhibitory concentration (µg/ml) and minimum fungicidal or bactericidal concentration (µg/ml) of oleo-gum resin of F.assafoetida*

Microorganisms	MIC (µg/ml)	MBC or MFC (µg/ml)
Bacillus subtilis	1562.5	>100000
Escherichia coli	1562.5	>100000
Staphylococcus aureus	781.3	25000
Saccharomyces cereviciae	195.3	100000
Candida albicans	390.6	50000
Aspergillus flavus	781.3	6250
Aspergillus parasiticus	390.6	125000

* The values in the table are an average of 3 experiment.

Results obtained followed by measurements of MIC and MBC indicated that oleo-gum resin of *F.assafoetida* exhibited significant (P< 0.05) antibacterial activity against tested bacteria and its effect on *S.aureus* was more than *B.subtilis* and *E.coli*. The MIC value of oleo-gum resin on both *B.subtilis* and *E.coli* was 1562.5 µg/ml which was more than MIC value of *S.aureus* (781.3 µg/ml). The MBC value of *S.aureus* was 25000 µg/ml and >100000 µg/ml for both *B.subtilis* and *E.coli.*

Among tested yeasts, MBC value of *C.albicans* was less than *S.cereviciae.* MIC value of *A.parasiticus* (390.6 µg/ml) was in lower concentrate than *A.flavus* (781.3 µg/ml) while MBC value of these fungi was more than *A.flavus.* The antiparasite, antifungal and antimicrobial activities of essential oil and extraction of different part of *F.assafoetida* plant such as roots, leaves, seeds have been studied by different researchers [Mohammadi et al., 2009 and Sitara et al., 2008 and Kunwar et al, 2010 and Rahman et al., 2008] but, there is not enough data about the direct effects of oleo-gum resin of *F.assafoetida* on pathogenic microorganisms. Results indicated that oleo-gum resin of *Ferula assafetida* exhibited significant (P<

0.05) antibacterial activity against tested bacteria and its effect on *S.aureus* was more than *B.subtilis* and *E.coli*. This finding is similar to the report of kunware et al (2010) on Effect of essential oil of *F.assafoetida* against various microbial strains of gram-positive and gram negative bacteria. They showed that essential oil of *F.assafoetida* have strong antimicrobial activity on *B.subtilis, S.aureus* and *C.albicans* [Kunwar et al, 2010]. Also antibacterial activity of essential oil of *F.assafoetida* on *S.aureus* and *S.epidermidis* was demonstrated by Sasikumar et al (2007) [Sasikumar et al., 2007]. Rahman and gul (2008) showed that the essential oil of seeds of *F.assafoetida* can inhibited the growth of wide range of bacteria such as *B.subtilis, S.aureus, E.coli, S.typhi and SH.flexnery* [Rahman et al., 2008].

According to Table 1 oleo-gum resin of *F.assafoetida* showed MIC of 781.3 µg/ml for *A.flavus* and 390.6 µg/ml for *A.parasiticus* while MFC values were 6250 and 125000 µg/ml respectively. Antifungal activities of essential oil of seed and gum of *F.assafoetida* against *A.flavus, A.parasiticus* and *A.niger* have been reported by Siddiqui *et al.* (1996) [Siddiqui et al., 1996]. Also Gowda et al (2004) found that assafoetida possesses moderate antifungal properties against *Aspergillus parasiticus* [Gowda et al., 2004]. Rahman and gul (2005) in their research investigated inhibitory effects of *F.assafoetida* oil on the asexual reproduction stage of five species of the foodborne mold aspergillus such as *A.flavus* and *A.parasiticus*. They showed that *F.assafoetida* oil inhibited all the three stage of asexual reproduction (spore germination, mycelia growth and spore formation) [Rahman and Gul, 2005].

Results of this study indicated that oleo-gum resin of *F.assafoetida* exhibited strong activity against *Saccharomyces cereviciae* and *Candida albicans* that this results is similar to report of Kamble and Patil (2008) on Effect of essential oil of *F.assafoetida* against *A.niger*, *S. cereviciae* and six species of *Candida* [Kamble and Patil, 2008]. Angelini et al (2009) demonstrated that methanolic extraction of oleo-gum resin of *F.assafoetida* has antifungal activity in higher concentrations [Angelini et al., 2009] but Naeini et al (2011) reported that ethanolic and acetonic extraction of *F.assafoetida* plant has weak effect on *Candida albicans* [Naeini et al., 2011]. Many researchers have been conducted for antifungal and antibacterial activities of essential oil and extraction of ferula in medium [Mohammadi et al., 2009 and Garg et al, 1980 Thyagaraja et al., 1996 and Yesodharan et al., 2007]. Comparison results of ten oleogum resin concentrations on the growth of *B.subtilis, E.coli and S.aureus* were showed in Figure 1. According to Figure 1 the inhibitory effect of oleo-gum resin of *F.assafoetida* on the growth of all microbial species increased significantly ($P<0.05$) as oleo-gum resin concentration increased. Growth of bacterial species in six initial concentrations decreased significantly ($P<0.05$) and its effect on *S.aureus* was more than two other bacterial species. The growth of bacterial species increased clearly in 195.3 µg/ml concentration which indicated that the oleo-gum resin of *F.assafoetida* in low concentrations had low effect on the growth of tested bacterial (Figure 1).

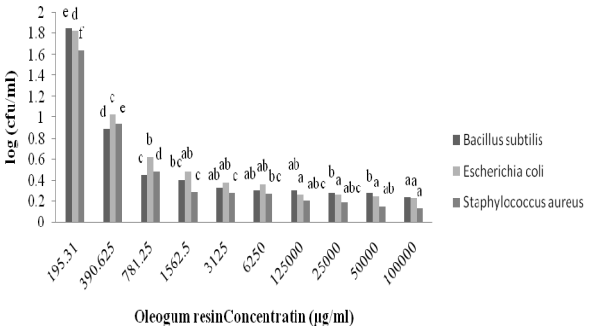

Figure 1: Effect of different concentration solution of oleo-gum resin of *F.assafetida* on the growth of *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus*. (n=3) -Data with the same letter for each oleo-gum resin concentrations are not significantly different ($p<0.05$) according to Duncan's multiple range test.

According to Figure 2 growth of yeasts decreased with increasing concentration of oleo-gum resin of *F.assafoetida* but its effect at all concentrations on *S.cereviciae* was more than *C.albicans.*

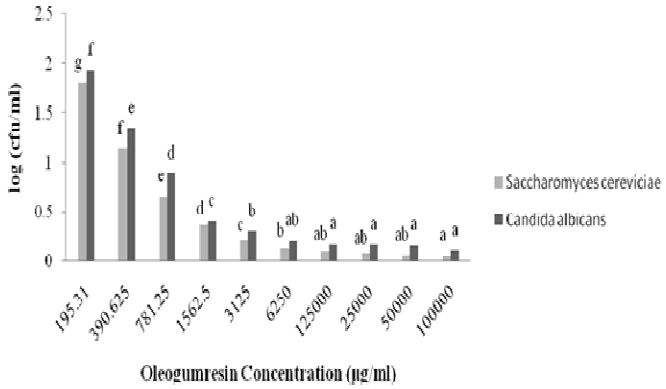

Figure 2: Effect of different concentration solution of oleo-gum resin of *F.assafetida* on the growth of *Saccharomyces cereviciae* and *Candida albicans.* (n=3)-Data with the same letter for each oleo-gum resin concentrations are not significantly different ($p<0.05$) according to Duncan's multiple range test

The oleo-gum resin of *Ferula assafetida* in high concentrations had the same effects on the growth of *A.flavus* and *A.parasiticus* but at lower concentrations (195.31 and 390.625 µg/ml) its effects on the growth of *A.flavus* was more than *A.parasiticus* (Figure 3)

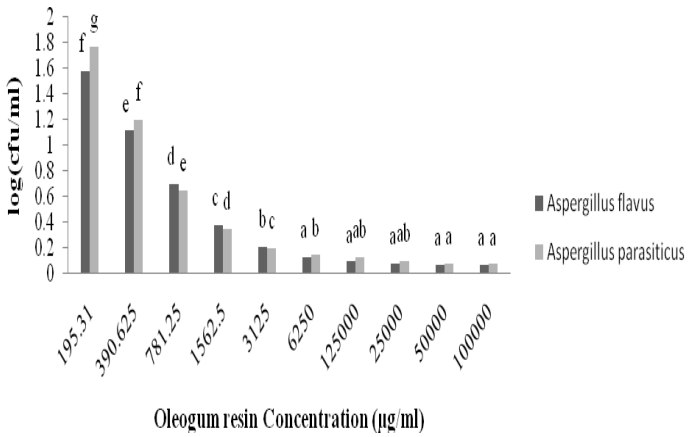

Figure 3: Effect of different concentration solution of oleo-gum resin of *F.assafetida* on the growth of *Aspergillus flavus* and *Aspergillus parasiticus*. (n=3)-Data with the same letter for each oleo-gum resin concentrations are not significantly different ($p<0.05$) according to Duncan's multiple range test

CONCLUSION

The results obtained in this study clearly indicate that the oleo-gum resin of *Ferula assafetida* has significant effect on growth of some bacterial and fungal species. Considerable pressure from world health organization to use natural preservations in foods and also consumer demand to reduce or eliminate chemically synthesized additives in their foods has led to a renewal of scientific interest in natural substances. This study has demonstrated that oleo-gum resin of *F.assafoetida*, already used in many parts of Iran for medical purposes, can strengthen the potential use of these plant in the future as alternative means to control bacterial and fungal contamination in stored foods and improve shelf life, quality and nutritional value of food commodities.

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