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Evaluation of Antimicrobial Activity of Essential Oil and Ethanolic Extract of 10 Medicinal Plants on *Rathayibacter tritici* and *Xanthomonas translucens*

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Abstract

Objective: Diseases caused by various drug-resistant strains in plants are increasing in many countries, so many efforts have been made to find new compounds as a suitable alternative to chemical drugs and pesticides. In this study, the antimicrobial effect of essential oils and ethanolic extracts of 10 medicinal plants were investigated on *Rathayibacter tritici* and *Xanthomonas translucens*.

Material and Methods: Alcoholic extracts of medicinal plants were extracted using a rotary apparatus. Two standard bacteria *R. tritici* and *X. translucens* were prepared from Persian Type Culture Collection. The minimum inhibitory concentration and the minimum inhibitory concentration of essential oils and ethanolic extracts of plants used at a concentration of 50 mg/ ml were determined by dilution in liquid medium on pathogens.

Results: Based on the results, the lowest inhibitory concentration of thyme essential oil was 6.25 ppm, which was inhibited by *R. tritici*, and the lowest concentration of *Hypericum perforatum* essential oil against *X. translucens* was 6.25 ppm. The lowest concentrations of essential oils of yew and fennel were 6.25 ppm, which were inhibited by both bacteria. *Rubia tinctorum* leaf essential oil in a concentration of 6.25 only inhibited *R. tritici* bacteria. The antibacterial properties of the essential oils of the studied plants were higher than the extract.

Conclusion: Essential oils of yew and oleander were the most effective against *R. tritici* and *X. translucens*, followed by thyme and rosemary against *R. tritici* and herring flower against *X. translucens*. Although the clinical use of ethanolic extracts and essential oils of the studied plants seems valuable due to side effects, but for the clinical use of essential oils and extracts, more research should be done on the mechanism of action of effective compounds of these plants on microbial agents.

Introduction

Undoubtedly, the use of medicinal plants has been the oldest human approach to the treatment of diseases and during the development of all human civilizations has always had a close relationship between man and plant and existence [1-4]. Due to drug resistance and side effects of antibacterial drug, chemical, scientific research approach to natural resources abundant in recent decades, including in several studies has proven antimicrobial effects of different plants [6-8].

Today, the use of medicinal products derived from medicinal plants is increasing [9,10]. Medicinal plants are reservoirs rich in the active ingredients of many drugs, which

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are mainly secondary metabolites of the plant [11,12]. Although these materials are mainly made by directing genetic processes, but their production in such a way that environmental factors cause changes in the growth of medicinal plants and also affect the quantity and quality of materials [13]. Therefore, due to the emergence of bacterial strains resistant to chemical drugs, it is necessary to try to find new antimicrobial agents. Today, research on new drugs derived from natural sources as a systematic way and has strategic and economic value in the world has become particularly important [14, 15]. However, the side effects of these compounds are less compared to chemical drugs.

Bacteria are widely used to cause infectious diseases in a variety of hosts, including domestic and wild animals, humans, and even some plants. Disease bodies are spreading in the environment through animal remains (carcasses and feces) and foods of animal origin [16].

R. tritici has been introduced as one of the two causative agents of wheat gum disease [17]. Significant symptoms of this disease are leakage of gum or yellow leachate on spikelets or wheat spikes [18]. This disease has been reported in Iran for the first time from Maragheh [19] and then from different parts of the country [20]. Isolates of pathogenic bacteria were isolated for the first time from spikes of gums with gum cluster symptoms from Ilam province and were identified as a species of Rathayibacter based on biochemical characteristics [21].

The genus *Xanthomonas* in the family *Xanthomonadaceae* is the only family in the order Xanthomonadales and belongs to the gamma-proteobacteria. Members of this genus are rod-shaped, motile, polar, aerobic, and chymoorganotrophic bacteria. The production of xanthomonadine and xanthan exopolysaccharide pigments is specific to bacteria of this genus and has not been reported in any other bacterium. Members of this genus are diseased plants and are divided into 20 species based on DNA homology [22, 23]. Bacteria of the genus Xanthomonas are one of the most important bacteria in crop diseases and are important causes of post-harvest corruption and economic damage. Meanwhile, *X. translucens* is one of the most important strains of the genus *Xanthomonas*, which causes tape spot disease in wheat. [24].

Considering that various studies have been done on the antimicrobial effect of essential oils and extracts of some plants [25] but on the other hand, in some cases, very little antimicrobial activity has been reported [26] and on the other hand, because Iran is climatic in terms of plant growth. The variety is very rich and most of the native plants of Iran have medicinal properties. Therefore, many plant extracts and their compounds have known antioxidant and antibacterial effects and they can be used as food preservatives or a suitable alternative to antibiotics [16]. Accordingly, in this research, the essential oil and ethanolic extract of plants were tried; Chicory (*Cichorium intybus* L), *Thymus vulgaris*, *H. perforatum*, *Lavandula spica*, *Taxus baccata*, *Anethum graveolens* dhi, Fennel (*Foeniculum vulgare*), *Bitter Melon*, *Nerium Oleander* and *R. tinctorum* on *R. tritici* and *X. translucens*.

Materials and Method

Bacteria studied:

Including *X. translucens* bacteria (ptcc 1473) and *R. tritici* bacteria (ptcc 1290) (Chr-Hansen Company, Denmark) that were obtaine from Persian Type Culture Collection. These bacteria were cultured in BHI medium at 37 ° C for 16-18 hours at least twice in a row and then mixed with sterile glycerin 5: 1 from the second culture. And stored in volumes of 500 µl in Ependref microtubes at -20 ° C (27).

Plant material:

Chicory leaves (Zagros, Galehdar), Thyme (Zagros, Galehdar), Hoofariqoon (Mazandaran, Behshahr), Lavender (Zagros, Galehdar), yew (Mazandaran, Behshahr), dill (Sistan), fennel (Sistan), Carla (Sistan), oleander (Sistan) and *R. tinctorum* (Sistan) were collected were collected and the species of them were determined in the botanical laboratory, f University of Zabol. The leaves were crushed in natural conditions in dry shade and then crushed. To prepare the extract, 40 g of dry plant powder was placed in half-liter Erlenmeyer flakes containing 200 ml of 96% ethanol.

The contents of the Erlenmeyer flask were mixed at room temperature for 24 hours with a shaker (130 rpm) and then filtered through Whatman 2 paper. The solvent was separated from the extract by a rotary apparatus using a vacuum pump (distillation in vacuum). The weighted extracts were then dissolved in DMSO solvent.

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Preparation of Essential Oil:

In order to obtain essential oil, first 100 grams of dry plant material of the aerial part (lateral branches with leaves) of each sample was ground for 1 minute using a laboratory mill (with 2800 watts of power and 25000 rpm). Then it was placed in Clevenger apparatus for 4 hours and its essential oil was extracted by water distillation method and dehumidified using sodium sulfate and kept in dark glass jars with impermeable air at 4 ° C until analysis [27, 28]. The amount of essential oil was calculated as a weight-weight percentage.

Preparation of Ethanolic Extract:

To prepare ethanolic extract, 10 grams of dried plant powder with 100 ml of 96% ethanol for 24 hours at room temperature is mixed by shaker (Pars Azma; Iran) at a speed of 130 rpm, then by Whatman No. 2 paper was flattened. The solvent was separated from the extract by a rotary apparatus (Heidolph; Germany) using a vacuum pump (vacuum distillation). The weighted extract was then dissolved in DMSO solvent. The obtained extract was stored in the refrigerator at 4 ° C until use in antimicrobial experiments [29].

Determining the MIC and the MBC of the prepared plant extract and essential oil

Sensitivity of bacterial strains to plant extracts was determined using CLSI methods and dilution method using 96 house microplate wells. For this purpose, 100 microliters of MHB was first added to each microplate well. Then, 100 microliters of diluted solution of each plant was added to the first well, and after mixing, 100 microliters were removed from the first well and added to the second well, and thus this process continued until the last well. 100 µl of the culture medium mixed with the extract was removed from the last well, and 10 µl of the microbial suspension containing 108 units/ml (equivalent to 0.5 McFarland) was added [30, 31].

The pellets were incubated at 37 °C for 24 hours. After 24 hours, the first well that prevented bacterial growth was considered the MIC. To ensure that bacteria did not grow in the clear wells (meaning that the bacteria were completely killed), 10 µL of the contents of each clear well was removed and transferred to the Müller-Hinton agar medium. If no growth was observed after 24 hours, the relevant well (equivalent to dilution of the extract used by the extract) was considered as the MBC of the extract. Also, standard strains of *R. tritici* and *X. translucens* were purchased from the collection of bacteria and fungi of the Scientific and Industrial Research Organization of Iran and the antibacterial properties of different species of medicinal plants studied in this study were investigated [30, 32]

Results

The results of this study showed that the extract of yew plant in a concentration of 12.5 ppm inhibited the two strains of *R. tritici* and *X. translucens*, while the lowest inhibitory concentration of fennel extract was 12.5 ppm and the bacterium *X. translucens* was inhibited. The highest inhibitory concentration of Hofariqon extract was 100 ppm, which was inhibited by *R. tritici*, and thyme extract was inhibited by 100 ppm *X. translucens* (Table 1).

The results of this study showed that the lowest inhibitory concentration of thyme essential oil was 6.25 ppm, which was inhibited by *R. tritici* and the lowest concentration of Hofaricon essential oil was 6.25 against *X. translucens*. The lowest concentration of yew-oleander essential oil was 6.25 in which two bacteria were inhibited. *R. tinctorum* leaf essential oil was inhibited at a concentration of 6.25 by *R. tritici* (Table 2).

The highest MBC of Hofariqoon plant extract was 200 ppm, which was inhibited by *R. tritici*. The ethanolic extract of thyme was inhibited at a concentration of 200 ppm of X. translucens (Table 3).

The lowest MBC of thyme, yew, oleander and rhubarb essential oils against *R. tritici* were 12.5 ppm and the essential oils of hoofaricon, yew, dill and fenugreek were inhibited by *X. translucens* at a concentration of 12.5 ppm (Table 4).

The highest MBC of chicory-*H. perforatum* essential oils was 50 ppm, which was inhibited by *R. tritici*, while the essential oils of chicory, thyme, thyme and carla were inhibited by *X. translucens* at 50 ppm. (Table 4).

Table 1. *MIC of ethanolic extract of medicinal plant on standard bacteria*

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Bacteria strain	*R. tinctorum*	*N. Oleande*	*B. melon*	*F. vulgare*	*A. graveolens dhi*	*T. baccata*	*L. spica*	*H. perforatum*	*T. vulgaris*	*C. intybus*
R. tritici	25	25	25	25	50	12.5	25	100	12.5	**50**
X. translucens	50	12.5	50	25	25	12.5	25	25	100	**50**

Table 2. *MIC of Medicinal Plant Essential Oil on Standard Bacteria*

Bacteria strain	*R. tinctorum*	*N. Oleande*	*B. melon*	*F. vulgare*	*A. graveolens dhi*	*T. baccata*	*L. spica*	*H. perforatum*	*T. vulgaris*	*C. intybus*
R. tritici	6.25	6.25	12.5	12.5	12.5	6.25	12.5	25	6.25	**25**
X. translucens	12.5	6.25	25	12.5	6.25	6.25	12.5	6.25	25	**25**

Table 3. *MBC of ethanolic extract of medicinal plant on standard bacteria*

Bacteria strain	*R. tinctorum*	*N. Oleande*	*B. melon*	*F. vulgare*	*A. graveolens dhi*	*T. baccata*	*L. spica*	*H. perforatum*	*T. vulgaris*	*C. intybus*
R. tritici	50	50	50	50	100	25	50	200	25	**100**
X. translucens	100	25	100	50	50	25	50	50	200	**100**

Table 4. *MBC of medicinal plant essential oil on standard bacteria*

Bacteria strain	*R. tinctorum*	*N. Oleande*	*B. melon*	*F. vulgare*	*A. graveolens dhi*	*T. baccata*	*L. spica*	*H. perforatum*	*T. vulgaris*	*C. intybus*
R. tritici	12.5	12.5	25	25	25	12.5	25	50	12.5	**20**
X. translucens	25	12.5	50	25	12.5	12.5	25	12.5	50	**50**

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Discussion

The lowest inhibitory concentration of thyme essential oil was 6.25 ppm, which was inhibited by *R. tritici*, and the lowest concentration of Hofaricon essential oil against *X. translucens* was 6.25 ppm. The lowest concentrations of essential oils of yew and fennel were 6.25 ppm, which were inhibited by two bacteria. *R. tinctorum* leaf essential oil in a concentration of 6.25 only inhibited *R. tritici* bacteria. The effect of rosemary extract and its microemulsion with aloe vera extract on Brucella bacteria was concluded and it was concluded that the microemulsion of rosemary extract had no inhibitory effect on the growth of Brucella strains. It has been reported that the lack of antibacterial effect of microemulsion can be associated with high dilution of the extract and low concentration of active ingredients [33]. However, in the present study, St. John's wort was one of the most effective plants with antimicrobial properties, which shows the different effects of plants against different bacteria.

The antimicrobial properties of the hydroalcoholic extract of 29 herbs on Escherichia coli and Staphylococcus have been evaluated and it has been concluded that the plant, thyme, yarrow and rosemary can be effective plants in clearing some bacteria, including Escherichia coli and *E. coli* [34]. In the present study, it was found that thyme essential oil was one of the most effective inhibitors against the activity of *R. tritici* bacteria.

Antimicrobial effects of *Leptogium saturninum*, *Ramalina peruviana* and *Punctelia borreri* extracts on *Xanthomonas campestris* were found and all extracts were effective against *X. campestris* and *S. aureus*, but in the case of *E. coli* and *B. cereus* only *R. peruvian* extract. It was effective and *P. aeruginosa* gram-negative bacteria also showed resistance to all extracts.

MIC results also showed that *Leptogium saturninum* and *Ramalina peruviana* extracts were effective on all three yeasts. However, licorice extract of *P. borreri* was effective only on *C. albicans* [35]. In the present study, of the ten medicinal plants studied, thyme, rhubarb, calendula, yew and oleander were the most effective against *R. tritici* and *X. translucens.*

Antibacterial effect of some plant essential oils on *Pseudomonas syringae* pv. Syringae and *R. tritici* have been studied in vitro and it has been concluded that plant species, essential oil concentration, bacterial type and dual and triple effects will have different effects against the studied bacteria. The highest antibacterial effect is related to the inhibitory aura of Sazil essential oil with an average of 13.3 mm for *E. coli* [36]. In the present study, it was found that out of 10 studied plants, some were effective against *R. tritici* (garden thyme and rhubarb) and some against *X. translucens* (herringbone), which indicates the different effects of plant species on microbial activity.

In recent years, the number of resistant pathogens has increased due to improper use of chemicals and synthetics. Existence of antioxidant and antiseptic properties in addition to therapeutic effects has been one of the factors in traditional medicine's attention to medicinal plants [37]. In addition, the presence of secondary compounds in medicinal plants has led to special attention in recent studies. In particular, the presence of antimicrobial compounds in medicinal plants has doubled the importance of these plants for the production of new and natural antibodies in the medical sciences. Therefore, scientists have prioritized the study of different parts of medicinal plants to discover new drugs of plant origin. Due to the fact that environmental factors cause changes in the growth of medicinal plants and the quality of the active ingredients in them, harvesting a medicinal plant is cost-effective when its effective ingredients have reached the desired level.

Harvesting medicinal plants at the wrong time not only reduces the yield; also, the harvested product will not be of good quality; because the function of the target organ as well as the amount of secondary metabolites of a medicinal plant are different in different stages of plant development [38].

Conclusion

Due to the very high adaptability of the bacterial genome to environmental conditions, antibiotics as the most important human tool to fight infectious diseases are becoming increasingly ineffective. Therefore, the introduction of new therapies or complementary therapies, including the use of inhibitory and lethal properties of medicinal plants against disease objects has become more and more necessary. In the present study, it was found that yew and fennel were the most effective plants against *R. tritici* and *X. translucens*, followed by thyme and rhubarb against *R. tritici* and herring flower against *X. translucens.*

Alternative or complementary treatments with alcoholic extracts of medicinal plants can be effective. However, in vivo

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testing is necessary to evaluate the possible toxicity of the extracts, to evaluate their properties and side effects, and to obtain appropriate concentrations.

Abbreviation

MHB: Müller-Hinton nutrient medium

MIC: minimum inhibitory concentration

MBC: minimum inhibitory concentration

Conflict of interest

None of the authors have any conflict of interest to declare.

Consent for publications

All authors approved the final manuscript for publication.

Availability of data and material

Data are available on request from the authors.

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