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The *In Vitro* Study of Antimicrobial Effect of Marigold (*Calendula officinalis*) Extract on Infectious Microorganisms

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Research Article

Abstract

Introduction: Infectious diseases have always been one of the important concerns of human and have continuously attracted the attention of a large number of various medical and laboratory professionals. On the other hand, treatment with antibiotics has other problems such as drug resistance and side effects, so the use of new herbal medicines with fewer side effects can be a great help in treating these types of infections. The objective of this study was to investigate the antibacterial activity of marigold (*Calendula officinalis*) extract on four reference strains including *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Pseudomonas aeruginosa*.

Materials and methods: In this study, the antimicrobial effects of methanolic extract of marigold plant were experimented on four above mentioned reference strains after extraction by Soxhlet method. Methanolic extract with concentrations of 20 mg/ml, 30 mg/ml, 50 mg/ml and 400 mg/ml was prepared by solvent dimethyl sulfoxide. Then, their antimicrobial effects were investigated using well diffusion and tubular dilution methods. The data were analyzed using Analysis of Variance (ANOVA) and Chi-square test at $P<0.01$.

Results: The results showed that the methanolic extract of marigold plant inhibit the growth of *S. aureus*, *B. cereus* and *E. coli*, which also increased their antimicrobial activity by increasing the concentration. As well as, the concentration of 1000 µg/ml essential oil of this plant leaves also showed an inhibitory effect on *S. aureus*, *B. cereus* and *E. coli*.

Conclusion: The results of this study showed that the extract of marigold plant has antimicrobial effect.

Keywords: Antibacterial; Herbal extract; Marigold flower.

1. Introduction

Plants have been still considered as a potential source of medicinal compounds. Traditionally, plants have been used to treat many diseases, especially infectious diseases, including diarrhea, fever and cold, as well as birth control and oral hygiene through the world [1]. Food contaminated with pathogenic microorganisms is often described as the primary source of many diseases in humans. The survival and growth of microorganisms in food products can spoil them and degrade their quality [2]. Nowadays, the incidence of a diverse drug resistance of pathogenic microorganisms has become an important challenge in both areas of human and veterinary healthcare. Therefore, there is a continuing need for identifying new antimicrobial compounds to minimize drug resistance of microorganisms [3,4]. Plant essential oils and extracts, which contain a variety of biological and physiological compounds, have a high potential for their application as new drug compounds in the field of health and treatment of human and animal diseases; and since antimicrobial, anti-cancer and antioxidant compounds and free radical removal factors, they have been identified as one of the most important sources of natural drug compounds [2,5]. Antimicrobial activities of plant essential oils have formed the foundation of many applications, such as the preservation of medicinal raw and processed foods [6]. Many people have greatly attempted to use the potential of antimicrobial activity of plants with increasing the number of resistant bacterial strains to various antibiotics. On the other hand, the emergence of resistant strains among gram-negative bacilli and gram-positive cocci such as Pseudomonas, Klebsiella, Enterobacter, Staphylococcus and Enterococcus has resulted in some problems in the treatment of infections caused by these bacteria [4]. Plant-derived antimicrobials eliminate bacteria with different mechanisms from antibiotics which this is clinically important in the treatment of infections caused by resistant microbial strains [7]. According to the re-approach for the use of herbal medicines and products, the study of the medicinal properties of endemic plants in each region is very important. Many studies have been conducted on extracts prepared from plants that have been collected randomly or in one of the above methods. These studies have further focused on the evaluation of antimicrobial activity, anti-worm activity, anti-viral activity, cytotoxic and mutagenic activities

as well as general pharmacological activities [8-13]. In this study, the antibacterial properties of methanolic extract and essential oil of marigold plant are investigated on some bacterial pathogens.

Marigold flower (*Calendula officinalis*) is an herbaceous, beautiful plant with a stem 20-50 cm in length. Its leaves are simple, long elliptical-shape, fluffy with jagged edges and a light brownish-green color. The large and beautiful capitols appear on its branching stems at 9:00-10:00 a.m. and they usually close at 4:00-5:00 p.m. depending on the temperature and humidity of its living environment [14]. This plant grows in the west of Iran, Herzvil, Masjed Soleiman and the southwest of Iran [15]. As well as, it is cultivated in countries of Germany, Australia, Austria, Switzerland and Syria as a medicinal plant [16]. In the past, marigold (*Calendula officinalis*) flowers were used for intestinal disorders, liver troubles, insect and snake bites. Nowadays, marigold flowers are known as a rejuvenating and soothing substance for seizure. For this reason, they are applied for asthma, cough, heart palpitations, insomnia, and anxiety to work. It is also recommended to use this plant to remove gastrointestinal inflammation and ulcers, and in its external use, its application on superficial body wounds, burns, colds, skin rashes, jaundice or impetigo and it is an effective medication for prevention of cancerous blood degeneration. Furthermore, it has a beneficial effect on patients with cancer who cannot be operated surgically [17]. The antitumor effect of this plant extract has been confirmed by tow mechanisms of cytotoxicity and lymphocytes' activation in various studies [8,18]. Due to the presence of various phytochemicals with significant antibacterial potential in the marigold plant, experimental studies are needed to determine the quality and extent of the effect of the above mentioned substances on pathogen microorganisms.

2. Materials and Methods

Fresh leaves and flowers of marigold (*Calendula officinalis*) plants were collected from Southwest Iran and the samples were botanically certified by the Department of Botany of Islamic Azad University of Ahar Branch. Water distillation method and Clevenger apparatus were used for essential oil extraction, so that 300 g of the plant dried powder was heated with 1 L distilled water in Clevenger apparatus. The collected essential oils were stored at 20°C after being dehydrated by sodium sulfate in dark and closed glass bottles. 60 g of dried plant powder with 300 ml methanol as solvent were placed to the Soxhlet Extractor for 8 h to prepare the methanolic extract. The solvent was slowly evaporated at 40°C using a rotary machine, and the concentrated extract was obtained. The extracts concentrated by 5% Dimethyl Sulfoxide (DMSO) were prepared with concentrations of 20, 30, 50 and 400 mg/ml for applying the determination tests of Minimum inhibitory concentration (MIC) and Disc diffusion. The microorganisms were prepared as lyophilized bacteria including *Bacillus cereus* (ATCC:1247), *Staphylococcus aureus* (ATCC:25923), *Pseudomonas aeruginosa* (ATCC: 27853) *Escherichia coli* (ATCC:25922) from the microbial collections of the University of Tehran. Microbial samples were recovered according to the standard method. Since the number of inoculated bacteria is one of the most important variables that influence the outcome of this study, the density of the microbial suspension of the inoculums should be standardized. For this purpose, 4-5 colonies were transferred to the culture medium of Muller-Hinton broth to prepare a microbial suspension from bacterial fresh and young culture to ensure the turbidity of microbial suspension prepared according to McFarland standard tube No. 0.5 (bacterial equivalent turbidity/ml). The microbial suspension with a turbidity equivalent to 0.5 McFarland was diluted to 0.01 to obtain the concentration of 1.5×10^6 bacteria/ml. In order to investigate the antimicrobial effect of methanolic extract, four concentrations of 20 mg/ml, 30 mg/ml, 50 mg/ml and 400 mg/ml from methanolic extract of the plant were prepared in 5% solvent DMSO. In this study, the antimicrobial activity of methanolic extract was investigated using Agar Well Diffusion and Dilution Test. In the well diffusion method, 500 ml microbial suspension 1.5×10^6 cfu/ml was transferred onto Muller-Hinton agar culture medium and it was cultured on three directions by sterile swab. Then, wells were produced in 6 mm diameter and the distance of 2.5 cm from each other at the agar surface. Subsequently, 100 µl of concentrations of 20, 30, 50 and 400 mg/ml from the methanolic extract was injected into each well.

The negative control was obtained by using a solution that was applied to dissolve the extracts (DMSO 5%) and the chloramphenicol antibiotic was used as a positive control. Then the plates were incubated at 35°C for 24 h and after the specified period of microbial cultures were measured in terms of formation or non-formation of zone of growth inhibition in mm. Growth MIC and MBC of methanolic extract were determined using the tubular dilution method. In this method, methanolic extract prepared from diluted series of 6.25, 12.5, 25, 50, 100 and 200 mg/ml used to determine MIC and in Muller-Hinton Broth. Then, 1ml active bacterial suspension 1.5×10^6 cfu/ml was added to each dilution. Beside the tubes, positive control (a culture medium containing bacteria without extraction) and negative control (a culture medium with no bacteria) were used. Finally, the tubes were incubated at 37°C for 24 h. After incubation time, tubes were examined for turbidity caused by inoculated bacterial growth and the last dilution in which no turbidity was observed (lack of growth) was considered as MIC. Subsequently, all tubes in which no bacterial growth was observed were sampled and minimum bactericidal concentration (MBC) was determined by culture in plate. Then the plates were incubated at 37°C for 24 h. The tube containing the lowest concentration of the extract, which was observed in the plate related to the lack of growth of the bacterium, was considered as the

MBC of the substance. The agar dilution method was used to confirm the antimicrobial property of the leaf essential oil. Thus, the concentration of 1000 mg/ml essential oil was prepared in DMSO in the culture medium of Muller-Hinton agar. The plates were placed at 25°C for 30 min. A microbial suspension was prepared at a concentration of 1.5×10^5 cfu/ml and it was inoculated on its own site. Next to each of the above plates, a plate was used as a control, which was only containing DMSO and a culture medium with no essential oil. Inoculated culture media were placed at 37°C for 24 h and then, they were assessed for growth or non-growth of bacteria. Each of the above experiments was repeated five times in order to reduce the experimental error. The analysis of variance test and chi-square was used to study the significant difference of results and the difference between the groups was determined at a significant level of $p<0.001$.

3. Results

The results of the effect of methanolic extract concentrations of marigold by the well diffusion method are presented in Table 1. Comparison of concentrations of 20, 30, 50 and 400 mg/ml methanolic extract by the well diffusion method on four strains of *S. aureus*, *B. cereus*, *E. coli* and *P. aeruginosa* showed that two bacteria of *S. aureus* and *B. cereus* have the highest microbial susceptibility to methanolic extract, and this inhibitory effect increased by increasing the concentration of methanolic extract on these two bacteria, which was observed as an increase in the zone of growth inhibition. Also, the results of the diameter of the zone of growth inhibition indicate that the inhibitory effects of methanolic extract of marigold plant were very low on the gram negative bacteria, so that they did not have any inhibitory effect on growth of *P. aeruginosa*. The concentrations of 400 mg/ml leaf methanolic extract showed a small inhibitory effect on *E. coli*. The values for the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) of the methanolic extract of marigold plant against the four studied bacteria are presented in Table 2. The results showed that the concentration of 25 mg/ml methanolic extract of marigold plant is effective on *S. aureus*. The lethal concentration of this extract on *B. cereus* was obtained to be 12.5 mg/ml. These results indicate that there is a significant difference between the tested bacteria in terms of the susceptibility to the marigold extract ($p<0.001$). In other words, the highest susceptibility to methanolic extract of marigold was observed in *B. cereus* and the least susceptibility to methanolic extract of marigold was considered in *P. aeruginosa*. The relevant experiments of the concentration of 1000 mg/ml leaf essential oil showed the inhibitory effect on *B. cereus*, *S. aureus* and *E. coli* and no inhibitory effect and growth reduction was observed on *P. aeruginosa*.

4. Discussion

Humans have long been using plants as dietary supplements as well as in the treatment of diseases. Currently, many plants are being annually investigated around the world in terms of therapeutic properties. A part of these researches focuses on determining the antimicrobial properties of medicinal plants. This has been confirmed due to the occurrence of problems such as microbial resistance and antibiotic complications. Low infectious doses of many food-borne pathogens require extensive research in the field of new pharmaceutical compounds with high bactericidal potential, which is very important to use oily compounds from plants to provide health and food safety in order to achieve this objective [19]. Regarding the fact that antibiotics affect pathogenic bacteria in very small amounts of micrograms, in the case of plants, it is also tried to identify the plants with a stronger antimicrobial activity by screening them, so that more favorable antimicrobial properties are benefited by using lower concentrations of them. Marigold flower has been widely used in traditional Iranian medicine as one of the most important traditional medicinal plants. This plant belongs to Asteraceae family, which

Table 1. The diameter of the zone of growth inhibition in mm of four bacteria strains of methanolic extract of marigold plant organs at different concentrations.

Positive Control	Negative Control	20	30	50	400	Extract Concentrations (mg/ml) / Strain of Bacteria
19 ± 0.92	--	--	8 ± 0.84	8 ± 0.92	18 ± 0.61	*Bacillus cereus*
26 ± 0.41	--	--	--	--	12 ± 0.43	*Escherichia coli*
22 ± 0.83	--	--	--	--	--	*Pseudomonas aeruguniosa*

Table 2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration of methanolic extract of marigold plant organs on the studied bacteria in *mg/ml*.

Extract Concentrations (mg/ml) / Strain of Bacteria	MIC	MBC
Staphylococcus aureus	12.5	25
Bacillus cereus	6.25	12.5
Escherichia coli	100	200
Pseudomonas aeruginosa	--	--

has been used in the treatment of a large number of diseases and in combination with homeopathic medicines; and its history in medicine dates back to the twelfth century. Even this plant has been used as a traditional medicine in diets [20]. The applicable parts of this plant are its leaf and flower and the most important therapeutic active substances include flavonoids, saponins, triterpenoids, carotenoids, sterol, cholesterol, vitamins A, organic acids, glaze, mucilage, gum, and albumin. In addition, the marigold flower has 0.2-0.4% essential oil, which its important substances include mentone, isomentone, gamma-terpinene, caryophyllene and tocopherol, polysaccharides, calenduline, α- and β-amirine acid and taraxasterol [13,21,22]. It is identified in this study that the methanolic extract of marigold plant inhibits the growth of gram-positive bacteria at concentrations around 30 mg/ml, while higher concentrations are required to affect gram-negative bacteria. As well as, the marigold essential oil has a significant inhibitory effect on *S. aureus*, *B. cereus* and *E. coli*. The results of this study showed the antibacterial activity of methanolic extract of marigold plant on gram-positive and gram-negative bacteria. The growth inhibitory effect of this extract begins at concentration of 30 mg/ml on gram-positive bacteria. The zone of growth inhibition significantly increases with increasing concentrations of the extract to 400 mg/ml. As well as, the results show that the effect of methanolic extract on gram negative bacteria is much weaker than that on the gram-positive bacteria, so that the concentration of 400 mg/ml leaf methanolic extract exhibits a poor inhibitory effect on *E. coli*. None of the concentrations had an inhibitory effect on *P. aeruginosa* due to the presence of cell wall lipopolysaccharides, which probably prevent the active ingredients of essential oil and extracts from reaching the cytoplasmic membrane of gram negative bacteria [23]. Generally, plant products result in cytoplasm granulation, cystoplasmic membrane rupture, inactivating or preventing the activity of intracellular and extracellular enzymes, and the cell wall collapse [24,25]. Thus, the majority of plant extracts have a lower inhibitory effect on gram-positive bacteria and gram-negative bacteria, among which *P. aeruginosa* is the most resistant bacterium to most plant extracts. Ghafari et al. [26] in a paper entitled "Evaluation of leishmanicidal effect of watery and ethanolic flowers *Calendula officinalis* extract on promastigotes of *Leishmania major* (MRHO/IR/75/ER) *in vitro*" showed that the marigold extract has a good leishmanicidal effect and it may be used to treat Leishmaniasis. Doustar et al. [27] in a study entitled "Study of apoptosis induced by *Calendula officinalis* extract on experimental colon carcinoma in rats" concluded that the marigold flower extract has an inhibitory effect on the expression of nucleus β-catenin protein in the cryptic misplaced cells in experimental colorectal cancer in rats. Ghasemi Pirbalouti et al. [22] in a paper entitled "Effect of jasmonic acid and salicylic acid on polyphenol and flavonoids in extract of *Calendula officinalis* L. flower" showed that the effects of jasmonic acid and salicylic acid spray were significant on carotenoids and polyphenol contents in marigold flower extract at 1% probability level and were significant on total flavonoid content at 5% probability level. Haroon et al. [18] in a study entitled "Evaluation of antioxidant and anti-glycemic properties of *Calendula officinalis* flower extract," showed that this plant has antioxidant activity and has an effective role in reducing the complications of diabetic disease. Alicia et al. [28] in a paper entitled "Investigating the effect of *Calendula officinalis* flower extract on acute and chronic oral brachilabases in Wistar rats," concluded that marigold has an anti-inflammatory effect. Eslami et al. [29] in a study entitled "Comparison of the effect of *Calendula officinalis* flower with cephalexin on bacteria isolated from cellulite patients," showed that gram positive bacteria such as Streptococcus and Staphylococcus were sensitive to the extract and gram negative bacteria such as Escherichia, Pseudomonas and Proteus were resistant to this extract, which the results of the present study are consistent with the results of the above-mentioned researchers.

5. Conclusion

From the present study, it can be concluded that there is the most antibacterial activity of marigold (*Calendula officinalis*) extract and essential oil on gram-positive bacteria, so that the active compounds contained in this extract has no growth inhibitory effect on *P. aeruginosa*, which has an external membrane with purines with very small pores. This extract can be considered as a natural herbal product according to the significant antibacterial effect of methanolic extract of marigold on pathogenic bacteria, especially gram-positive samples that contribute to the development of various malignant and nosocomial infections.

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References

[1] Mitscher LA, Drake S, Goliapudi SR, et al. (1981). A modern look at folkloric use of anti- infective agents. *J Natl Prod.* **50:** 1025-1040.

[2] Celiktas OY, Kocabas EEH, Bedir E, et al. (2007). Antimicrobial activities of methanol extracts and essential oils of *Rosmarinus officinalis*. Depending on location and seasonal variations. *Food Chem.* **100:** 553-559.

[3] Gragg GM, Newman DJ, Sander KM. (1997). Natural products in drug discovery and development. *J Nat Prod.* **60:** 52-60.

[4] Oussalah M, Caillet S, Saucier L, et al. (2007). Inhibitory effects of selected plant essential oils on the growth of four pathogenic bacteria: *E. coli*, *Salmonella typhimurium*, *Staphylococcus aureus* and *Listeria monocytogenes*. *Food Control.* **18:** 414-420.

[5] Hussain AI, Anwar F, Sherazi STH, et al. (2008). Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depend on seasonal variations. *Food Chem.* **108:** 986-995.

[6] Bozin B, Mimica-Dukic N, Simin N, et al. (2006). Characterization of the volatile composition of essential oils of some lamiaceae spices and the antimicrobial and antioxidant activities of the entire oils. *J Agric Food Chem.* **54:** 1822-1828.

[7] Eloff JN. (1999). It is possible to use herbarium specimens to screen for anti-bacterial components in some plants. *J Ethnopharmacol.* **67:** 355-360.

[8] Khafagi IK, Deweder A. (2000). The efficiency of random versus ethno-directed research in the evaluation of Sinai medicinal plants for bioactive compounds. *J Ethnopharmacol.* **71:** 365-376.

[9] Rao KS. (1996). Antibacterial activity of some medicinal plants of papua New guinea. *Int J Pharmacogn.* **34:** 223-225.

[10] Naqvi SAH, Khan MSY, Vohora SB. (1991). Antibacterial antifungal and antihelminthic investigations on Indian medicinal plants. *Fitoterapia.* **62:** 221-228.

[11] Vlietinck AJ, Van Hoof L, Totte J, et al. (1995). Screening of hundred Rwandese medicinal plants for antimicrobial and antiviral properties. *J Ethnopharmacol.* **46:** 31-42.

[12] Alkofahi A, Batshoun R, Owais W, et al. (1997). Biological activity of some Jordanian medicinal plant extracts. Part II. *Fitoterapia.* **68:** 163-168.

[13] Khalid KA, El-Ghorab AH. (2006). The effect of presowing low temperature on essential oil and chemical composition of *Calendula officinalis L. Journal of Essential Oil Bearing Plants*. **9:** 32-41.

[14] Zargari A. (1990). Medicinal plants, Tehran University Press, Amir Kabir Press. **2:** 250-253.

[15] Zargari A. (1990). Medicinal plants, Tehran University Press, Amir Kabir Press. **5**: 398-401.

[16] Omid Beigi R. (2009). Production and processing of Medicinal plants, 5th edn, Nazarestan Ghods Razavi. **2:** 299-305.

[17] Erfani H. (2005). One hundred plants and thousands of treatments, 5th edn of Tehran, Coin Publishing. 362-364.

[18] Haroon A, Ibrar Kh, Abdul W. (2012). Antiglycation and antioxidation properties of *Juglans regia* and *Calendula officinalis*: Possible role in reducing diabetic complications and slowing down ageing. *Journal of Traditional Chinese Medicine.* **32:** 411-414.

[19] Gill AO, Holley RA. (2006). Disruption of *E. coli*, *Listeria monocytogenes and Lactobacillus sakei* cellular membranes by plant oil aromatics. *Int J Food Microbiol.* **108:** 1-9.

[20] Zitterl-Eglseer K, Sosa S, Jurenitsch J, et al. (1997). Anti-edematous activities of the main triterpendiol esters of marigold (*Calendula officinalis* L.). *J Ethnopharmacol.* **57:** 139-144.

[21] Pour Navaie MN. (2007). Introduction of Iranian Anticancer Medicinal Plants. 1st edn. Research Institute of Forests and Rangelands of Iran. **2:** 135-137.

[22] Ghasemi Pirbalouti A, Jafarian Dehkordi M, Poorhakim Davoodi R, et al. (2011). The effect of *Hypericum scabrum* on the hematologic factors, body weight and temperature in mice. *Journal of Herbal Drugs.* **1:** 25-31.

[23] Duraipandiyan V, Ayyanar M, Igancimuthu S. (2006). Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. *BMC Complement Altern Med.* **6:** 35.

[24] Caccioni DLR, Guzzardi DM, Renda A. (2000). Relationships between volatile components of citrus fruit essential oil and antimicrobial action on *Pencillium digitatum* and *Penicillium italicum*. *Int J Food Microbiol.* **88:** 770-775.

[25] Kraft K, Hobbs C. (2004). Poket guide to herbal medicine. New York: Thieme Stuttgart. 61-62.

[26] Maspi N, Ghafarifar F, Bahrami AM, et al. (2010). Evaluation of leishmanicidal effect of watery and ethanolic flowers *Calendula officinalis* extract on promastigotes of *Leishmania major* (MRHO/IR/75/ER) *in vitro*. *SJIMU.* **18:** 28-33.

[27] Doustar Y, Mohajeri D. (2010). Immunohistochemical study of the effect of *Calendula officinalis* of β-catenin protein expression in experimental colon cancer. *Gastroenterology.* **15:** 126-31.

[28] Alicia L, Viviana B, Isbel G, et al. (2011). Acute and sub chronic oral toxicities of *Calendula officinalis* extract in Wistar rats. *Exp Toxicol Pathol.* **63:** 387-391.

[29] Eslami G, Taheri S, Ayatollahi SA, et al. (2011). Effects of *Calendula officinalis* extract on bacteria isolated. *Res Med.* **34:** 214-218.