Original Article

Antimicrobial Activity of *Scabiosa Olivieri* Extract and Its Effect on TNF-α and IL-1 Expression in Human Peripheral Blood Cells (PBMCs)

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Abstract

Background & Objective: Infectious diseases caused by bacteria and fungi have affected billions of people worldwide. Throughout human history, infectious diseases have been the leading cause of death and disability. Infectious diseases today account for one-third of all deaths in the world .The general objective of this study was to investigate the antimicrobial activity of *Scabiosa Olivieri* on gram-positive, gram-negative bacteria and fungi, as well as to study its anti-inflammatory properties by investigating the factors of human IL-1 and TNF-α, which ultimately led to the introduction of an antimicrobial agent and new anti-inflammatory drugs with a natural and inexpensive source.

Materials & Methods: First, the extract of the plant was prepared by maceration. Then, the antimicrobial properties of this extract on *E. coli*, *Staphylococcus aureus*, and *Candida albicans* were investigated by MIC. Also, the effect of this extract on the expression of IL-1 and TNFα in human peripheral blood mononuclear cells was evaluated by ELISA.

Results: *Scabiosa Olivieri's* extract significantly showed anti-inflammatory properties and has antimicrobial activity against *Escherichia coli* and a mild antimicrobial activity against *Staphylococcus aureus*. But the antifungal property was not observed.

Conclusion: It seems that *Scabiosa Olivieri's* extract can be used as an anti-inflammatory and antimicrobial agent. However, the effects of its use in the in vivo environment and the chemical analysis of its constituent compounds require further research.

Keywords: Scabiosa Olivieri, PBMC, anti-inflammatory effect, antimicrobial activity, MIC, IL-1, TNF-α

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Introduction

Infectious diseases caused by bacteria and fungi affect billions of people around the world. During the history of humanity, contagious diseases have been the leading cause of death and disability. Today, infectious diseases account for one-third of all deaths in the world; the World Health Organization estimates that nearly 50,000 people die every day due to infectious diseases worldwide (1).

The synthesis of a large number of antibiotics over the past three decades has led to a negligence of the great danger of bacterial resistance, and the bacteria become more resistant by chromosomal changes and the exchange of genetic elements through plasmids and transposons, which will lead to a crisis in the future (2). The presence of multi-drug resistant pathogens has endangered the effectiveness of

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many antibiotics. This condition is known worldwide as a global concern and justifies further research to explore antimicrobial agents of natural origin, including herbs (3).

On the other hand, many common diseases in the world, such as atherosclerosis, type 2 diabetes, and Alzheimer's, have important pathophysiological inflammatory compounds. In these diseases, the exact identity of the inflammatory stimuli is unknown or, if known, cannot be resolved. Therefore, treating these diseases is the target of inflammatory responses (4). Chronic inflammatory diseases are not directly induced by the autoimmune process and are the most common diseases of aging and the most significant health threats (5).

Also, the relationship between inflammation and cancer has long been discovered, and there is a strong link between inflammation and the incidence of cancer-causing lesions in various tissues (6). For example, a 14% increase in prostate cancer risk due to prostate inflammation (7), 25% increased risk of bowel cancer due to ulcerative colitis (8), and 10 to 20 times the risk of pancreatic cancer due to pancreatic inflammation (9). Therefore, the presence of inflammation facilitates or induces carcinogens. In the past, some anti-inflammatory drugs such as aspirin and non-steroidal anti-inflammatory drugs (NSAIDs) have been used to prevent and treat cancers such as intestine, esophagus, breast, lung, and bladder (10).

With increasing antibiotic resistance in the world, the discovery of new antimicrobial agents has become one of the most important challenges and goals of scientists around the world. On the other hand, with an ever-increasing rise in inflammatory and cancerous conditions, the study of anti-inflammatory and anti-cancer agents is regarded as the most attractive subject of study for scientists.

Evaluation of *Scabiosa* herb extract in terms of antimicrobial and anti-inflammatory properties is in line with both global goals to solve the most important health risks in today's society.

The genus *Scabiosa* belongs to the Dipsacaceae family and has 80 species in the world; 43 species are found in Europe and the rest in Asia and Africa (11). Several species of this plant are used in traditional medicine to treat many disorders. *S. atropurpurea* L. was used as a diuretic (12). Another example is the use of *S. succisa* to treat bronchitis, bronchial pneumonia, influenza, and asthma. It is also recommended as an external medicine for the treatment of skin diseases and stomach ulcers (13).

Phytochemical studies of several species of Scabiosa revealed that the plants belong to this genus, have triterpenes (14), triterpene glycosides (15), terpene saponins (16), iridoids (17), monoterpenoids glucoindole alkaloids (18), and flavonoids (19,20). It has been discovered that this genus has anti-cancer agents, such as carotenoids in *S. atropurpurea* (21) and hentriacontane in *S. comosa* (22). Recently, the antioxidant activity of *S. comosa* and *S. tschilliensis*, which are used in traditional Chinese medicine for liver disease, has been investigated (23).

The general objective of this study was to investigate the antimicrobial activity of *Scabiosa olivieri* on gram-positive bacteria, gram-negative bacteria and fungi, and its anti-inflammatory properties by measuring IL-1 and TNF-α cytokines.

Materials & Methods

Plant collection and extraction procedure

The plant was obtained from the suburbs of Qom city, and all part of *Scabiosa* plant was sliced and dried at room temperature and wholly crushed to powder. The 150g powder of the plant was added to 500 ml of 97% ethanol as a solvent and then placed on a shaker at room temperature for 48 hours. The crude extract was filtered using Whatman No. 1 filter paper and the solvent was eliminated using a rotary evaporator. Finally, the prepared extract was kept in 4º C until further use.

Antimicrobial test

Evaluation of minimum inhibitory concentration (MIC)

The antibacterial activity of the extract was performed on *Escherichia coli* (ATCC: 25922), *Staphylococcus aureus* (ATCC: 25923), and *Candida Albicans* (ATCC: 10231) prepared from the Pasteur Institute. The antimicrobial activity of *Scabiosa Olivieri* extract was utilized by the microdilution method according to CLSI protocol (24). 100µl of the extract was added at a concentration of 300mg/ml, and after mixing, 100µl of the first well was removed and added to the second well. This process continued to the final wells to provide different dilutions of the extract. Then 10 µl of microbial suspension equivalent to half McFarland was poured into each well and incubated at 37 ° C for 24 hours

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and examined for turbidity. As a control, ciprofloxacin (0.1mg/ml), tetracycline (0.1 mg/ml) and fluconazole (0.25 mg/ml) were used for *Escherichia coli*, *Staphylococcus aureus* and *Candida Albicans* respectively. For positive control, the medium containing microbe and negative control, the DSMO solution was used. The first non-turbid well was reported as the minimum inhibitory concentration (MIC) in mg/ml.

Evaluation of minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC)

MBC and MFC were also determined according to the MIC results. To assess MBC, the wells in which the bacteria were not grown were sampled by sterile swab and cultured on Mueller Hinton agar and incubated for 24 hours at 37°C and for MFC, each of the concentrations of the plant extract in which the Candida Albicans did not grow, were incubated in a Sabouraud dextrose agar medium for 48 hours at 29°C. All tests were repeated three times. The lowest concentration of the extract where the bacteria or fungi did not grow was reported as MBC and MFC respectively.

Preparation of peripheral blood mononuclear cells (PBMC)

For this purpose, blood samples were taken from 10 healthy people in EDTA-containing tubes. These people should be entirely negative for the use of alcoholic beverages, drugs, as well as inflammatory (acute or chronic) and infectious disease for up to 3 weeks before sampling. Peripheral blood cells were isolated using Ficoll, then counting and viability of the cells was performed.

MTT assay

The MTT assay is a colorimetric assay for assessing cell metabolic activity. NAD(P)H-dependent cellular oxidoreductase enzymes may reflect the number of viable cells present. These enzymes are capable of reducing the tetrazolium dye MTT to its insoluble formazan, which has a purple color. For this purpose, 2×10^5 PBMCs were incubated with different concentrations of Scabiosa for 48 hours, then MTT and 2-propanol were added and optical density (OD) was read at 570 nm by ELISA reader. Finally, by plotting the curve, IC50 (concentration of extract that causes the death of 50% of the cells) was calculated.

Measurement of TNF-α and IL-1 concentration by ELISA

In order to secrete cytokines, PBMCs were initially stimulated by killed bacteria (Escherichia coli and Staphylococcus aureus). Then, 2×10^5 of these cells were incubated with two concentrations close to IC50 (4 and 8 mg / mL) of the extract for 48 hours and 5% CO2. After collecting supernatant, to determine the concentration of TNF-α and IL-1, optical density (OD) of the samples was read at 450 nm by ELISA reader.

Statistical analysis

The results were analyzed by SPSS software, ANOVA, and Tukey's test. Quantitative data were reported as mean ± SD (± SD) and the significance level was considered to be $P<0.05$.

Results

Evaluation of MIC and MBC of bacteria

To investigate the antibacterial properties of the plant, a serial dilution of 150 mg/ml to 2.34

Table 1. Results of antimicrobial activity of the extract

Extract concentration (mg/mL)	150 mg/mL	75 mg/mL	37.5 mg/mL	18.75 mg/mL	9.37 mg/mL	4.68 mg/mL	2.34 mg/mL	Positive control	Negative Control (Bac+antibiotic)	Positive control (Bac+ DSMO)
Escherichia coli	-	-	-	-	-	+	+	+	-	+
Staphylococcus aureus	-	-	-	-	-	-	+	+	-	+

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mg/mL was prepared and loaded in the microplate wells. The results of this test are shown in Table 1. MIC for Escherichia coli and Staphylococcus aureus were reported as 9.37 mg/mL and 4.68 mg/mL, respectively.

MBC results are also measured and shown in Table 2. MBC values for Escherichia coli and Staphylococcus aureus were 18.75 mg / mL and 9.37 mg / mL, respectively. To verify the accuracy of these findings, the bacteria were cultured in a selective culture medium and then the plates were treated with the above concentrations. After 24 hours of incubation, no growth was observed.

Evaluation of the MIC and MFC levels of fungi

The antifungal activity of the extract was also performed by microdilution, and dilution series (from 300 mg/mL to 4/4 mg / mL) and the results are shown in Table 3. No antifungal activity was observed in the above concentrations.

In order to ensure the results, all wells were cultured in Sabouraud Dextrose agar medium and after 48 hours incubation at 29°C, cultures were examined. All of them had a negative result and Candida albicans had grown in all cases.

MTT assay

MTT assay was used to find the appropriate concentrations of the extract for treatment with PBMCs, and the results are shown in Fig. 1. Levels of 2, 4, 6, 8, 10 and 12 mg/mL of extract were used for MTT assay. As the concentration of the extract increases, the percentage of live cells is reduced. Based on this test, levels of 4 mg/mL and 8 mg/mL of extract were used for treatment with PBMCs. The results showed that more than 50% of cells (IC50) in these concentrations could survive and function. The results are shown in Chart 1.

Table 2. MBC results of tested bacteria

Bacteria Species	Strain	MIC (mg/mL)	MBC (mg/mL)
Escherichia coli	ATCC: 25922	9.37 mg/mL	18.75 mg/mL
Staphylococcus aureus	ATCC: 25923	4.68 mg/mL	9.37 mg/mL

Table 3. MIC results for Candida albicans

Extract concentration (mg/mL)	300 mg/mL	150 mg/mL	75 mg/mL	37.5 mg/mL	18/75 mg/mL	9.37 mg/mL	4.68 mg/mL	Positive control	Negative control
Candida Albicans	+	+	+	+	+	+	+	+	-

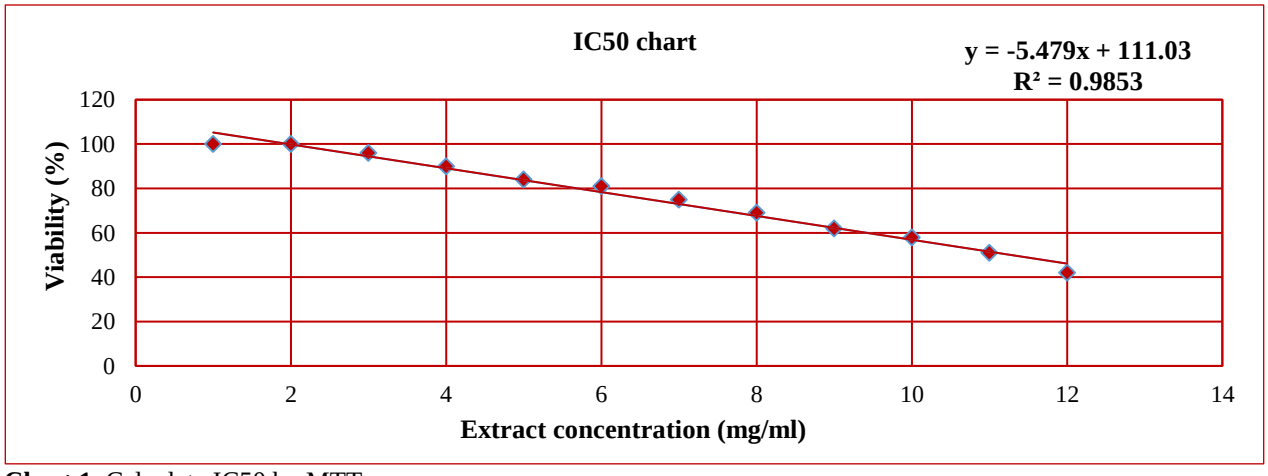


Chart 1. Calculate IC50 by MTT assay

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Interleukin 1 and TNF-α Assay

The results showed that interleukin 1 and TNF-α concentration significantly decreased ($p < 0.001$) in the wells treated with the extract, indicating anti-inflammatory properties. Also, the amount of inhibition of cytokine secretion at 4 mg/mL extract concentration was approximately equal to the level of 8 mg/mL and did not show a significant difference (Chart 2 and 3). In these figures, untreated PBMCs is a blank, meaning that these cells were not stimulated with bacteria and extracts. In other wells, the cells were irradiated with Staphylococcus aureus and Escherichia coli bacteria, resulting in high levels of interleukin 1 and TNF-α secretion. In other wells, the cells were treated with or without the stimulant and extract (4 and 8 mg/mL), which showed a significant decrease in cytokine secretion in both cases ($p < 0.001$). This result indicates the anti-inflammatory properties of the extract.

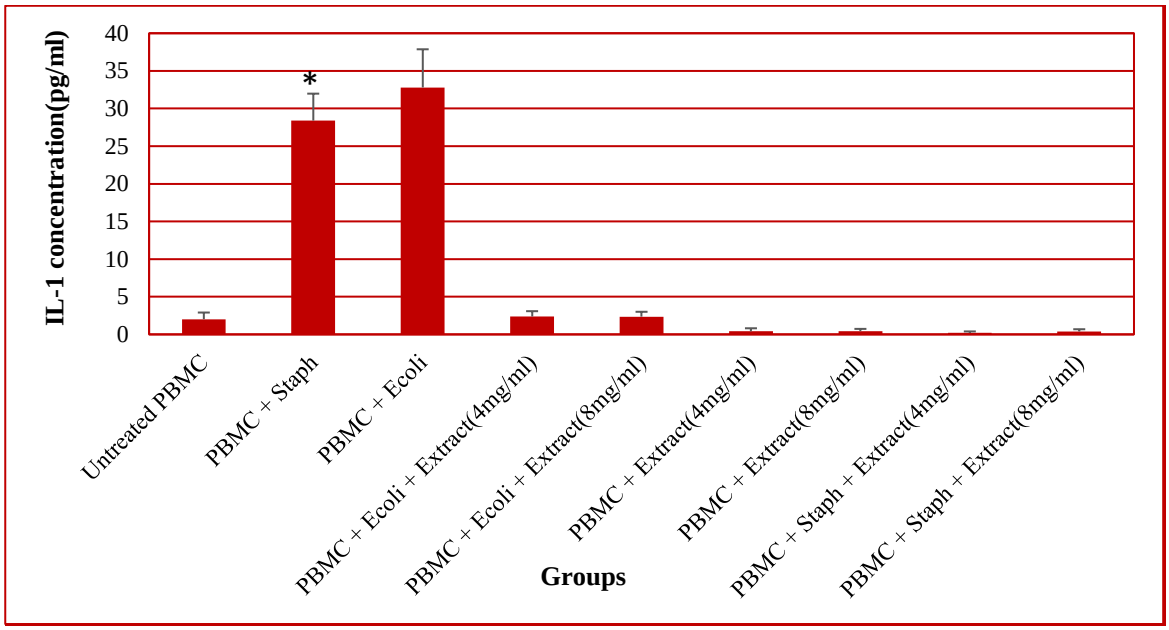


Chart 2. Interleukin 1 concentration in different cell groups.
*: Significantly different in comparison with other groups

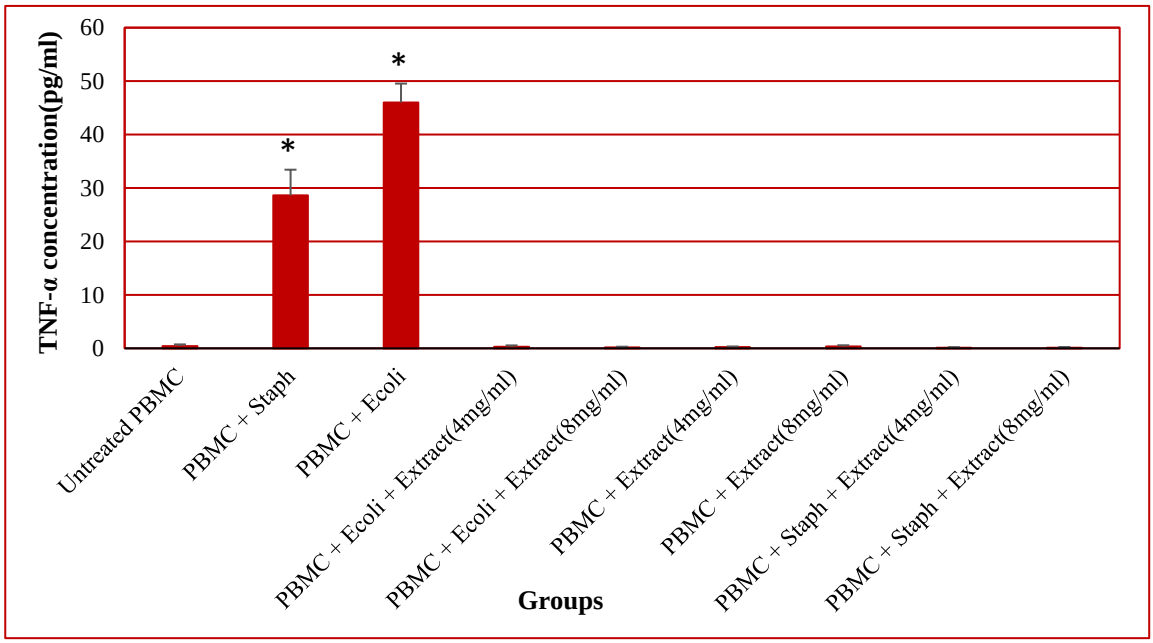


Chart 3. TNF-α concentration in different cell groups
*: Significantly different in comparison with other groups

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Discussion

In recent years, with the increasing elderly population and the presence of resistant strains of bacteria, finding new antimicrobial agents and the treatment of inflammatory diseases are two major challenges for human society (25,26). On the other hand, as it is known, traditional medicine has a special place in the treatment of diseases, and the use of herbs in traditional and local conditions is of particular importance (27). Traditional medicine covers most of the therapeutic needs of at least 80% of Africa's population. As such, these therapies include all aspects of physical, psychosocial, and social needs. The use of plants and natural compounds as medicinal sources in these countries or countries such as China and India, dates back to 4,000 to 5,000 BC, especially the Chinese who have been pioneering the use of these valuable resources (28).

Ethnobotany, whose history dates back to many years ago, in many cases, has been the subject of traditional mediccal issues. Many countries, whether in the Third World or developed countries, are expanding this issue in their health systems. Considering these discussions can be the foreground and the basis for discovering many of the unknown issues about natural compounds (29). Subsequently, many ethnopharmacological studies have been carried out to determine the safety, productivity, and discovery of new plants in recent years (30), while many medicinal plants and antibiotic-based herbal products have become significant sources of potent drugs (31).

Taking into consideration the subjects mentioned above and in line with other studies that have been carried out worldwide on various species of *Scabiosa*, the aim of this study is to evaluate the antimicrobial and anti-inflammatory properties of *Scabiosa most interesting*.

Antimicrobial activity of this extract was performed on three different microorganisms such as Escherichia coli (Gram-negative bacteria), *Staphylococcus aureus* (Gram-positive bacteria) and *Candida albicans* as a fungus (Tables 1 to 3). According to the results of this study, this extract had the most antimicrobial effect on Staphylococcus aureus but did not show any antifungal properties. These findings are consistent with previous studies that have been carried out on other members of the *Scabiosa* species, indicating that the antibacterial property of this plant is different in other plant species.

However, Ríos and Recio suggested that MIC results of higher than 1000 mg/mL of crude extract or 100 mg/mL of extracted compounds cannot be considered as antimicrobial, and MIC of 100 mg/mL for crude extracts and 10 mg/mL is desirable for extracted compounds(32), but Fabry et al. showed that MIC values less than 8000 mg/mL could be considered as a potent antimicrobial agent(33). Based on this definition, the *Scabiosa Olivieri* extract showed antibacterial properties especially on Staphylococcus aureus (MIC: 4/68 mg / mL). These results are consistent with another study by Christopoulou et al. which showed that *Scabiosa hemitia* had antimicrobial activity on some bacterial species, especially *Staphylococcus aureus* (34). Another study by Hlila et al. on *Scabiosa Arnaria* showed that this plant has the highest susceptibility to *Escherichia coli*, but in our study, the antimicrobial activity of the extract on *E. coli* was weaker (MIC: 9/37 mg/ml) (35).

In the case of antifungal activity, higher concentrations of extract were used compared to bacteria. Results showed that antifungal activity was not observed in these concentrations.

To investigate the anti-inflammatory activity of the extract, the present study showed that the ethanolic extract of *Scabiosa Olivieri* had an anti-inflammatory effect. In this study, due to the presence of luteolin in the *Scabiosa* family (36), we examined the anti-inflammatory properties of this extract. This plant contains flavonoids that these polyphenol compounds have analgesic and anti-inflammatory properties (37). Flavonoids are natural polyphenol compounds that inhibit glutamate NMDA receptors and reduce intracellular calcium and decrease the activity of nitric oxide synthase and calcium-dependent phospholipase A2, which ultimately show anti-inflammatory effects by reducing the production of nitric oxide and prostaglandins. Flavonoids also reduce the production of prostaglandin E from arachidonic acid by inhibiting cyclooxygenase enzymes in response to inflammatory stimuli. Because prostaglandins originate from arachidonic acid and contribute to inflammation and exacerbation of pain (38,39) Scabiosa Olivieri flavonoids probably play a role in anti-inflammatory activity.

Conclusions

In traditional medicine, the use of herbs is particularly essential in the treatment of diseases.

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Many developing and developed countries are expanding this field of science in their health systems. This study also showed that the extract of *Scabiosa Olivieri*, like other members of this family, has anti-inflammatory properties due to the presence of luteolin and polyphenolic compounds and therefore can be a suitable candidate for the treatment of various inflammatory diseases.

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Conflict of Interests

The authors report no conflicts of interest in this work.

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مقاله پژوهشی

بررسی فعالیت ضد میکروبی عصاره گیاه *Scabiosa Olivieri* و تأثیر آن بر بیان TNF-α و IL-1 در سلولهای خونی محیطی انسان (PBMC)

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چکیده

زمینه و هدف: بیماریهای عفونی ایجاد شده توسط باکتریها و قارچها میلیاردها انسان را در سراسر جهان تحت تاثیر خود قرار داده است. در طول تاریخ بشریت، بیماریهای عفونی عامل اصلی مرگ و میر و معلولیت بوده است. امروزه بیماریهای عفونی عامل یک سوم تمام مرگها در دنیا می باشند. هدف کلی این مطالعه بررسی گیاه دارویی *Scabiosa Olivieri* از نظر خاصیت ضد میکروبی بر روی باکتری‌های گرم مثبت، گرم منفی و قارچ و نیز مطالعه خاصیت ضدالتهابی آن با بررسی فاکتورهای IL-1 و TNF-α انسانی می‌باشد که در نهایت منجر به معرفی یک عامل ضدمیکروبی و ضدالتهابی جدید با منشا طبیعی و ارزان شود.

مواد و روش‌ها: ابتدا عصاره گیاه به روش ماسراسیون تهیه گردید و سپس به منظور بررسی خواص ضدمیکروبی، رقت‌های متفاوتی از عصاره این گیاه بر روی سه میکروارگانیسم اشرشیا کلای، استافیلوکوکوس اورئوس و کاندیدا آلبیکانس انجام شد همچنین تاثیر این عصاره بر روی سلول‌های تک هسته‌ای خون محیطی و اندازگیری فاکتورهای التهابی IL-1 و TNF-α بوسیله کیت الایزا مورد بررسی قرار گرفت.

نتایج: عصاره گیاه *Scabiosa Olivieri* به‌طور معنی‌داری خاصیت ضدالتهابی از خود نشان داد و همچنین خاصیت ضد میکروبی فعالی بر روی باکتری اشرشیا کلای و ضدمیکروبی متوسطی بر استافیلوکوکوس اورئوس دارد. اما خاصیت ضدقارچی مشاهده نشد.

نتیجه‌گیری: عصاره این گیاه پتانسیل استفاده بعنوان یک عامل ضدالتهابی و نیز ضدمیکروبی را داراست که البته بررسی اثرات استفاده آن در محیط in vivo و نیز آنالیز شیمیایی ترکیبات تشکیل دهنده آن نیازمند انجام تحقیق‌های بیشتری می‌باشد.

کلمات کلیدی: Scabiosa Olivieri، PBMC، خاصیت ضدالتهابی، فعالیت ضدمیکروبی، MIC, TNF-α , IL-1

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