International Journal of Molecular and Clinical Microbiology 11(2) (2021) 1556-1563

International Journal of
Molecular and Clinical Microbiology

The antibacterial, antioxidant and cytotoxicity effects of *S. officinalis* in an in vitro study

Saeed Akbari[1*], Fatemeh Zaboli[2]

1. Department of Parasitology, School of Medicine, Babol University of Medical Sciences.
2. Microbiology Department, Ayatollah Amoli Branch, Islamic Azad University of Amol, Amol, Iran.

ARTICLE INFO

Article history:
Received 04 September 2021
Accepted 30 October 2021
Available online 1 December 2021

keywords:
Antioxidant and cytotoxicity;
Salvia officinalis;
Staphylococcus aureus;
Klebsiella pneumonia;
Escherichia coli;
Pseudomonas aeruginosa

ABSTRACT

Natural plant products are the best candidate for antimicrobial and antioxidant properties and are the suitable alternative for chemical drugs. This study aimed to examine the antimicrobial effect of ethanol extract of *S. officinalis* on *S. aureus*, *E. coli*, *K. pneumoniae* and *P. aeruginosa* and its comparison with antibiotic discs of ciprofloxacin, ceftriaxone and gentamicin. In this experimental study the ethanolic extract of *S. officinalis* was extracted by maceration method and the concentrations of 1.9, 3.9, 7.8, 15.6, 31.2, 62.5, 125, 250, 500 and 1000 µg/ml were obtained. Standard microbial strains of *S. aureus*, E. *coli*, *K. pneumoniae* and *P. aeruginosa* were purchased from pasture Institute and the amount of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts was determined using micro dilution method. Antioxidant and cytotoxicity were evaluated using Diphenyl-1-Picrylhydrazyl (DPPH) Radical Scavenging Capacity and MTT assays. The ethanol extract of *S. officinalis* had different effects on *S. aureus*, *E. coli* and *P. aeruginosa* strains and inhibition zone diameter of 32.66, 10.83 & 10.6 mm were observed respectively. *S. officinalis* had an inhibitory effect on all of the studied bacteria except *K. pneumoniae* and this effect was higher in *S. aureus* bacteria. Also, the inhibition zone diameter of *S. officinalis* extracts was exceptionally higher in *S. aureus* compared to ceftriaxone and gentamicin. Moreover the ethanol extract of *S. officinalis* showed acceptable antioxidant and no cytotoxicity effects. Our results indicated that *S. officinalis* extracts had the greatest antibacterial effect on the gram-positive bacteria. Although the inhibition zone diameter of *S. officinalis* extracts was exceptionally higher in *S. aureus* compared to ceftriaxone and gentamicin.

1. Introduction

Bacteria are one of the most important causes of infectious diseases with a large influence on public health (Roberts and Buikstra, 2019). Tuberculosis, Anthrax, Tetanus, Leptospirosis, Pneumonia, Cholera, Botulism, Pseudomonas Infection, MRSA Infection, *E. coli* Infection, Meningitis, Gonorrhea, Bubonic Plague and Syphilis are the most deadly bacterial infections that made widespread epidemics in mammals, including humans (Roberts and Buikstra, 2019). Drug resistance is a worrying phenomenon that is unfortunately developing and has been reported in all types of microorganisms including bacteria, parasites, fungi and viruses and has a very high prevalence rate in various bacterial species (Elmi et al., 2020; Esboei et al., 2018; Yarahmadi et al., 2016). Drug resistance has developed against many drugs, one of the

*Corresponding authors: Saeed Akbari, PhD
Email address: Saeedakbari.2281@gmail.com

most famous of which is penicillin (Pourhajibagher et al., 2016).

Staphylococcus aureus, *Klebsiella pneumonia*, *Escherichia coli*, *Pseudomonas aeruginosa* ate the most important bacteria with high prevalence rate. Pulmonary infections, catheter-related bacteremia and urinary tract infections are the main clinical manifestation of *S. aureus*, *K. pneumoniae* happen in the lungs, where they cause necrosis, inflammation, and hemorrhage within the lung tissue. *E. coli* cause serious food poisoning, meningitis, septic shock and urinary tract infections. *P. aeruginosa* can cause septicaemia, bacteraemia mainly in Immunocompromised Hosts.

The resistance of microorganisms to treatment with specific drugs which was formerly was effective is called as Antibacterial resistance. These occurrences permitting infections to persist heightened the hazardous of clinical manifestations and maybe cause death. In actual fact, the mortality rate for patients with resistant bacteria is two times more than patients with same infections caused by sensitive bacteria (Pourhajibagher et al., 2016; Pourhajibagher et al., 2012). Drug resistance bacteria such as *Shigella* species and *Mycobacterium tuberculosis* has been directly associated to more than one million deaths yearly (WHO, 2014) (Pourhajibagher et al., 2012). According to the reports of Centers for Disease Control and Prevention (CDC, 2013) drug resistant bacteria with *Clostridium difficile*, *Enterobacteriaceae* Spp., and *Neisseria gonorrhoeae* are the primary cause of more than 23,000 deaths and 2 million serious infections each year in the United States (U.S.) (McGann et al., 2016).

Thousands of medicinal plants with different mechanisms of action have been reported as anti-microbial agents (Elmi et al., 2020; Fakhar et al., 2015). In recent years, many compounds have been extracted from medicinal plants that have had extraordinary effects. For example, drugs such as artimizinin (Su and Miller, 2015) and ivermectin (Tambo et al., 2015) are isolated plant-based drugs that have won the Nobel Prize, which shows the importance of natural compounds in the treatment of infections. Salvia spp. has been used as traditional plant worldwide for its anti-bacterial, anti-malarial (Pertiwi et al., 2021), anti-fungal (Nasrollahi et al., 2011), antimutagenic, anti-inflammation and anti-oxidant effectiveness (Deng et al., 2017). *S. officinalis* is one of the most effective genuses of *Salvia spp.,* which showed many therapeutic effects and mostly attributed to polyphenols. Current study is aimed to evaluate the antibacterial effects of *S. officinalis* against some conventional microbial pathogens.

2. Materials and Methods

2.1. Diphenyl-1-Picrylhydrazyl (DPPH) Radical Scavenging Capacity Assay

The antioxidant effects of the *S. officinalis* ethanolic extract was assessed using the standard method described by Uyory et al, 2020. In brief, the concentrations of 1.9, 3.9, 7.8, 15.6, 31.2, 62.5, 125, 250, 500 and 1000 µg/ml were added to 1.8 ml of a 0.004% methanolic solution of DPPH and the absorbance of the solution was measured at 517 nm until the reaction reached a plateau. After prepare a calibration curve by 0.01, 0.015, 0.02, 0.025, 0.03, 0.035 mg/l of DPPH by a linear regression, the percentage inhibition of DPPH by the extracts of phenolic compounds was calculated using the below formula (Choe et al. 2020):

$$\text{Inhibition (\%)} = \frac{Abs\,(blank) - Abs\,(sample)}{Abs\,(blank)} \times 100$$

Ascorbic acid and Trolox were used as positive control.

2.2. Cytotoxicity test

The cytotoxicity and/or growth inhibitory effects of the extracts were evaluated for J774 Macrophages cell lines. The cells were cultured in DMEM Medium (Gibco BRL, Invitrogen, Carlsbad, CA, USA), supplemented with 10% fetal calf serum (FCS; PromoCell, Heidelberg, Germany) and 1.0% Penicillin/Streptomycin mixture (Pen/Strep, 10,000 IU/mL, FCS; PromoCell) at 37 C for 72h. 100 µl of the medium with 1×10^4 cells were added to each well in 96-well plate, 10 µl of the each extract were then added to wells and a untreated well were considered as negative control. 60 µl of the

MTT solution were added and incubated at 37∘C

for 15 min, in a humidified atmosphere of 95% air/5% CO2 under dark. After incubation time,

the absorbance of the supernatant were assessed using an ELISA automatic microplate reader (SLT, Austria) at 570 nm, with a reference wavelength of 620 nm (Chabra et al., 2019).

2.3. Plant Material and Preparation of Extracts

Aerial parts of *S. officinalis* were collected in June and December of 2018 from mountainous areas of Amol city, northern Iran and the genus and species of the plant were realized by Dr. Mohammad Azadbakht with the herbarium No: 331-99-MAZUMS.Pharmacology school. The plants were dried at room temperature under shade situation and the ethanol extract were separated using maceration technique. The dried parts of the plants were grinded using Electric shredder and 500 g of the plant were added to the 3000 ml of the ethanol and stored at 37 C for 72h. After incubation time, the solution were filtered by whatman filter paper (No. 1) and then dried under vacuum using a rotary evaporator (Company). Finally the extracts were kept at 2 - 8°C until future use (Golami et al., 2016).

2.4. Bacteria strains

Escherichia coli (ATCC 25922), *Klebsiella pneumoniae* (ATCC 7881), *Pseudomonas aeruginosa* (ATCC 27853), and *Staphylococcus aureus* (ATCC 25923) strains were prepared from pasture institute, Iran and then were cultured in Muller intone agar (MHA) (Merk Germany) at 37°C and used as stock cultures. Several colonies of each bacterium were harvested by a sterile loop and inoculated into a test tube containing 5 ml of MHA medium, and then the medium was incubated at 37 °C for 24 hours to allow the bacteria to grow. Then the turbidity of the tube was matched with the turbidity of 0.5 McFarland tube (1.5×10^8 cfu / ml) and equalized.

2.5. *Agar-well diffusion assay*

Six mm diameter holes were made in MHA medium using a sterile pasteurizer pipette. Then, the bacterial sample was transferred and spread evenly with sterile swab. Ciprofloxacin, gentamicin and ceftriaxone as positive controls, plant extracts in different concentrations (80 µl) were added to each well. One well was untreated as negative control. The antibacterial effects were evaluated by measuring the diameter of the halos in millimeters using an electric caliper. All tests were carried out in triplicate and the average results of the report were calculated (Adeyemi et al., 2018).

2.6. MIC and MBC

To calculate the MIC and MBC concentration, the concentrations of 1.9, 3.9, 7.8, 15.6, 31.2, 62.5, 125, 250, 500 and 1000 µg/ml of the *S. officinalis* ethanolic extract were used. Determination of minimal inhibitory concentration (MIC) were assessed by the broth microdilution test and determined as the lowest concentration that prevented a change in resazurin color as bacteria growth indicator. The minimum bactericidal concentration (MBC) was calculated by the lowest concentration of the treatment yielding negative subcultures after incubation at 37 ºC for 24 h. All the tests were performed in triplicate (Pourhajibagher et al., 2014).

2.7. Statically Analysis

The data were analyzed using one-way ANOVA, with a Dunnett's multiple comparison tests and GraphPad Prism, version 5.02 (GraphPad Software, San Diego, CA, USA).The statical differences less than < 0.05 were considered as significant.

3. Results

3.1. Antioxidant Activities

The results of DPPH method indicated that The antioxidant activity of *S. officinalis* extracts was dose dependent and the concentration of 1000 µg/ml showed higher antioxidant activity than other concentrations (P=0.003) but lower than Ascorbic Acid as standards control (P=0.073). the antioxidant capacity (IC50) of vitamin C as standard control was 13.198 µg / ml and the IC50 of the concentrations of 31.2, 62.5, 125, 250, 500 and 1000 µg/ml of ethanolic extract were 34.21, 43.76, 54.28, 58.32, 63.60 and 69.84 µg/ml, respectively (Fig. 1).

3.2. Cytotoxicity effects

The cytotoxicity activity of *S. officinalis* extracts was evaluated by the MTT method and the results revealed that all concentrations of the *S. officinalis* extracts had similar cytotoxic

effects to normal Saline on J774 cell lines (P=0.0821).

3.3. Antimicrobial activity

In current work, the concentrations of 1.9, 3.9, 7.8, 15.6, 31.2, 62.5, 125, 250, 500 and 1000 µg/ml of the *S. officinalis* ethanolic extract were assessed for its anti-bacterial effects. According to the table and figure 1, the ethanolic extract of *S. officinalis* had best effects against *S. aureus* with the MIC and MBC of 3.9 and 7.8 mg/ml, respectively. The antibacterial effects of *S. officinalis* on *E. coli* and *P. aeruginosa* were significantly better than control groups (P=0.001). *K. pneumonia* was the most resistant strain to ethanolic extract of *S. officinalis* with the MIC and MBC concentration of >1000 µg/ml.

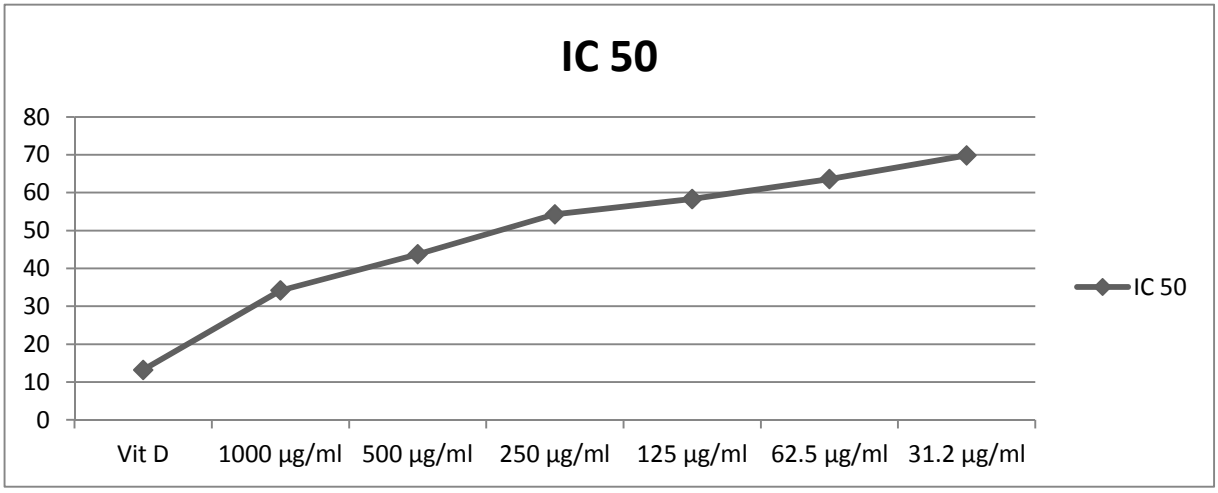

Fig. 1: The Antioxidant capacity (IC50) of different extractions of *S. officinalis* and Vit D as standard control.

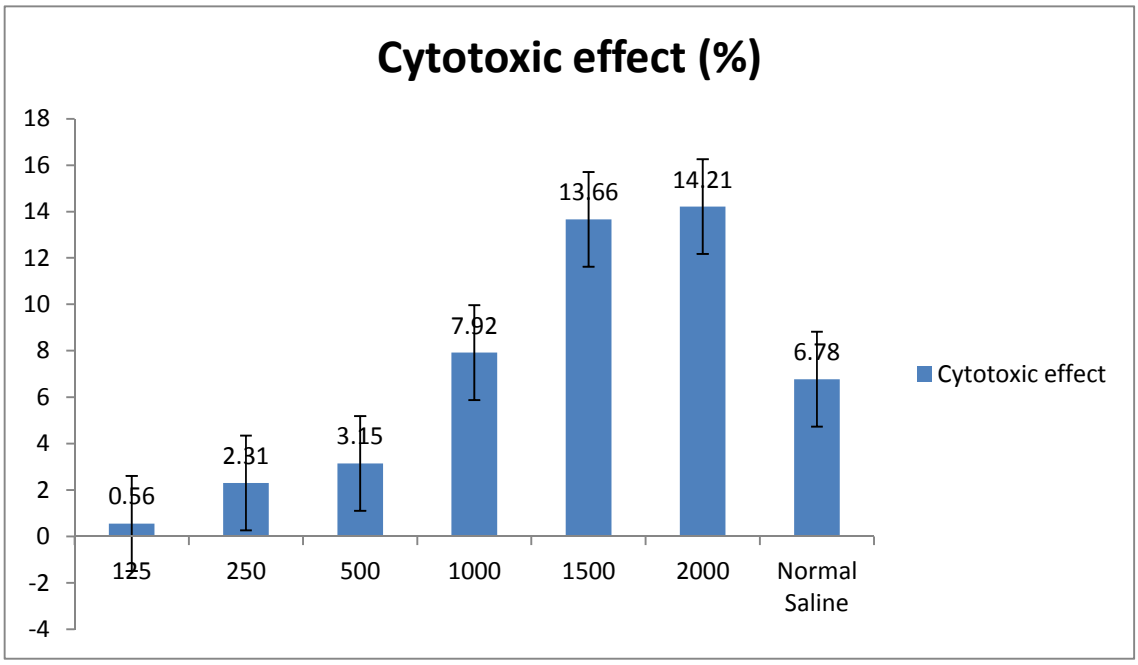

Fig 2. The Cytotoxic effect of different extractions of *S. officinalis* on J774 cell lines.

Table 1. The average diameter of non-growth halo (mm), MIC and MBC of *S. officinalis* for *S. aureus, E. coli, P. aeruginosa, K. pneumoniae* in vitro.

Concentration (µg/ml)	Average diameter of non-growth halo (mm)						MIC	MBC	P-value
	1000	500	250	125	62.5	31.2			
S. aureus	32.66±6.23	29.66±7.58	28±6.5	25.11±4.59	22.83±3.44	20.10±6.32	3.9	7.8	0.001
E. coli	10.83±3.81	7.66±2.43	7±1.5	3.64±1.21	0.0	0.0	125	125	
P. aeruginosa	10.61±4.52	8.82±1.66	6.80±3.41	3.16±0.94	0.0	0.0	125	250	
K. pneumoniae	3.21±1.75	1.37±0.68	0.0	0.0	0.0	0.0	>1000	>1000	

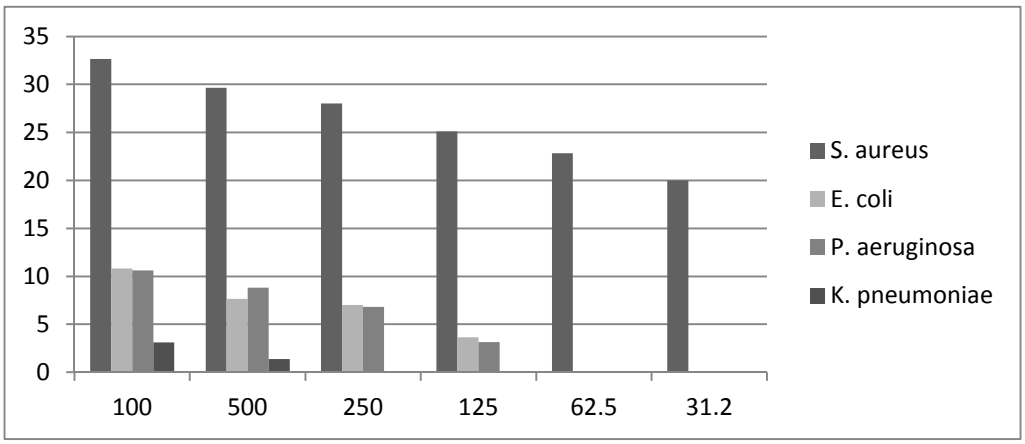

Fig 3. The average diameter of non-growth halo (mm), MIC and MBC of *S. officinalis* for *S. aureus, E. coli, P. aeruginosa, K. pneumoniae* in vitro.

4. Discussion

In this study, the antibacterial effect of *S. officinalis* on *S. aureus, E. coli, P. aeruginosa, K. pneumoniae* strains were investigated in vitro and the results showed that this plant, as mentioned in other experimental studies, was very effective. The results of this study showed that the ethanolic extract of *S. officinalis* in all concentrations had the best effect on *S. aureus*. Also, the effectiveness of this extract had moderate effects on *E. coli* and *P. aeruginosa*, but the amount of antibacterial effect on *K. pneumoniae* was not acceptable.

Chemical composition and morphological structure of the membrane of the bacteria is significantly associated to the effectiveness of the treatments (Elhidar et al., 2019). *S. aureus* is the only gram-positive bacterium in this study and the rest are gram-negative, so we can say that *S. officinalis* has better effects against gram-positive pathogens than gram-negative bacteria. However, the reason why *K. pneumoniae* is more resistant among gram-negative bacteria has not yet been determined and needs further study. In a study conducted by Kamatou GP et al., (2009), it's revealed that gram-positive bacteria are more sensitive to essential oils in comparison to gram- negative bacteria (Kamatou et al., 2009).

The effectiveness of these compounds in this study was dose and time dependent, so that with increasing dose and time, the amount of antibacterial effect of this compound also increases. *S. officinalis* is one of the most traditional remedy in the world and recently many investigations were carried out to find new biological effects for this plant. In some studies, the constituents of this plant have been studied and the results have shown that effective compounds such as terpenes/terpenoids, alkaloids, poly acetylenes, glycosidic

derivatives, steroids, fatty acids, carbohydrate, and phenolic compounds are present in this plant (Ghorbani and Esmaeilizadeh, 2017). Based on the many published works, antimicrobial, anti-inflammatory, antioxidant, anticancer, anti-nociceptive, hypolipidemic, hypoglycemic and antidementia effects were reported for *S. officinalis* (Kolac et al., 2017; Su et al., 2018; Su et al., 2019).

Et-Touys et al., (2016) evaluated the anti-bacterial and anti-leishmanial effects of the ethanol, n-Hexane and Methanol extracts of *S. officinalis* in vitro and reported an acceptable anti-bacterial and anti-leishmanial effects (Et-Touys et al., 2016). In 2012, Olgica. Stefanovic et al, revealed an effectiveness of *S. officinalis* extracts on *E. coli*, *S. aureus*, *P. aeruginosa*, *Bacillus Subtilis*, *Enterobacter cloacae*, *K. pneumonia* and *Proteus mirabilis* with the MIC of 0.25, 0.125, 0.25, 0.25, 0.25, 0.25 and 17.5 mg/ml (Stefanović and Comic, 2012).

The present study was a confirmation of previous studies and has once again proved that the plant *S. officinalis* has appropriate antibacterial effects. However, antimicrobial effects alone cannot cause this drug to be used as an antibacterial treatment. It is important to note that not all plants are safe, and some plants can even cause immediate death. Therefore, in addition to antimicrobial effects, it is necessary to study the toxic effects of natural compounds. In this study, the toxic effects of *S. officinalis* on the intestines of macrophage cells were investigated and the results showed that this plant has no toxic effects even at the highest concentrations.

Conclusion

The results of current study indicated that *S. officinalis* plant has acceptable antibacterial against *S. aureus*, *E. coli*, *P. aeruginosa*, *K. pneumonia* and antioxidant effects and also no toxic effects of this plant have been reported. According to these results, it seems that by conducting supplementary studies on this plant and examining the various extracts and fractions of this plant, it was introduced as a natural antibacterial drug.

Acknowledgment:

This study was carried out in Islamic Azad University, Ayatollah Amoli branch and the authors would like to express their deep thanks to all staff in pharmaceutics laboratories.

Conflict of Interest:

The authors declared that there are not any conflicts of interest.

Refereces

Adeyemi AI., Vincent OI., Olujenyo OM (2018). Phytochemical screening and antifungal activity of Chromolaena odorata extracts against isolate of Phytophthora megakarya using agar-well diffusion method. Asian Journal of Medical and Biological Research. 4(2):7-13

Chabra A., Rahimi-Esboei B., Habibi E., Monadi T., Azadbakht M., Elmi T., Akhtari J., Fakhar M., Naghshvar F (2019). Effects of some natural products from fungal and herbal sources on Giardia lamblia in vivo. Parasitology. 146(9):1188-1198

Choe U., Whent M., Luo Y., Yu L (2020). Total phenolic content, free radical scavenging capacity, and anti-cancer activity of silymarin. Journal of Food Bioactives. 10(1): 53-63.

Deng S., Ansari S., Ilkit M., Rafati H., Hedayati MT., Taghizadeh-Armaki M., Nasrollahi-Omran A., Tolooe A., Zhan P., Liao W (2017). In vitro antifungal susceptibility profiles of 12 antifungal drugs against 55 Trichophyton schoenleinii isolates from tinea capitis favosa patients in Iran, Turkey, and China. Antimicrobial agents and chemotherapy. 61(2): e01753-16.

Elhidar N., Nafis A., Kasrati A., Goehler A., Bohnert JA., Abbad A., Hassani L., Mezrioui N-E (2019). Chemical composition, antimicrobial activities and synergistic effects of essential oil from Senecio anteuphorbium, a Moroccan endemic plant. Industrial crops and products. 130:310-315.

Elmi T., Esboei BR., Sadeghi F., Zamani Z., Didehdar M., Fakhar M., Chabra A., Hajialiani F., Namazi MJ., Tabatabaie F (2020). In Vitro Antiprotozoal Effects of

Nano-chitosan on Plasmodium falciparum, Giardia lamblia and Trichomonas vaginalis. Acta Parasitologica. 66(1): 36-52.

Esboei BR., Mohebali M., Mousavi P., Fakhar M., Akhoundi B (2018). Potent antileishmanial activity of chitosan against Iranian strain of Leishmania major (MRHO/IR/75/ER): In vitro and in vivo assay. Journal of vector borne diseases. 55(2):111-115.

Et-Touys A., Fellah H., Mniouil M., Bouyahya A., Dakka N., Sadak A., Bakri Y (2016). Screening of antioxidant, antibacterial and antileishmanial activities of Salvia officinalis L. extracts from Morocco. Microbiology Research Journal International. 16(5):1-10.

Fakhar M., Chabra A., Rahimi-Esboei B., Rezaei F (2015). In vitro protoscolicidal effects of fungal chitosan isolated from Penicillium waksmanii and Penicillium citrinum. Journal of Parasitic Diseases. 39:162-167.

Ghorbani A., Esmaeilizadeh M (2017). Pharmacological properties of Salvia officinalis and its components. Journal of Traditional and Complementary Medicine. 7(2):433-440.

Golami S., Rahimi-Esboei B., Mousavi P., Marhaba Z., Youssefi MR., Rahimi MT (2016). Survey on efficacy of chloroformic extract of Artemisia annua against Giardia lamblia trophozoite and cyst in vitro. Journal of parasitic diseases. 40:88-92.

Kamatou G., Viljoen A., van Vuuren S., Balwanth P., Gosai R (2009) Variation in Essential Oil Composition of Boswellia Carterii Birdw. and Its Antimicrobial Activity. African Journal of Traditional, Complementary and Alternative Medicines. 3(11): 443-444

Kolac UK., Ustuner MC., Tekin N., Ustuner D., Colak E., Entok E (2017). The anti-inflammatory and antioxidant effects of Salvia officinalis on lipopolysaccharide-induced inflammation in rats. Journal of medicinal food. 20:1193-1200

McGann P., Snesrud E., Maybank R., Corey B., Ong AC., Clifford R., Hinkle M., Whitman T., Lesho E., Schaecher KE (2016). Escherichia coli harboring mcr-1 and blaCTX-M on a novel IncF plasmid: first report of mcr-1 in the United States. Antimicrobial agents and chemotherapy. 60:4420-4421

Nasrollahi A., Pourshamsian K., Mansourkiaee P (2011). Antifungal activity of silver nanoparticles on some of fungi. 1(3): 233-239.

Pertiwi AK., Habiburrohman M., Debby Y., Triatmoko B., Nugraha AS (2021). Ethnopharmacology and Computer-Aided Tandem Protocol to Search for Antimalarial Agents from Indonesian Medicinal Plants: HAP Inhibitor. Nusantara Science and Technology Proceedings. 12(2): 9-16.

Pourhajibagher M., Hashemi FB., Pourakbari B., Aziemzadeh M., Bahador A (2016). Antimicrobial resistance of Acinetobacter baumannii to imipenem in Iran: a systematic review and meta-analysis. The open microbiology journal. 10:32.

Pourhajibagher M., Nasrollahi M., Eini MI., Rahimi Esboei B (2014). Prevalence of β-lactamase Enzyme in Isolated Bacteria in Patients with Nosocomial Infections, Skin and Wound infections from Imam Khomeini Hospital of Sari Province, 2010. Journal of Mazandaran University of Medical Sciences. 23:261-265.

Pourhajibagher M., Nasrollahi M., Musavi S., Rahimi-Esboei B., Ghorbani Pashakolaei A (2012). Drug resistance in Mycobacterium tuberculosis isolates to isoniazid and rifampin. Journal of Babol University of Medical Sciences. 14:66-72

Roberts CA., Buikstra JE (2019). Bacterial infections. In: Ortner's Identification of pathological conditions in human skeletal remains. Elsevier. pp 321-439

Stefanović O., Comic L (2012). Synergistic antibacterial interaction between Melissa officinalis extracts and antibiotics. Journal of applied pharmaceutical science. 2:1-5

Su X-Z., Miller LH (2015). The discovery of artemisinin and the Nobel Prize in Physiology or Medicine. Springer. 58(11): 1175–1179.

Su XD., Guo RH., Li HX., Ma JY., Kim YR., Kim YH., Yang SY (2018). Anti-

allergic inflammatory components from Sanguisorba officinalis L. Bioorganic & medicinal chemistry letters. 28:2210-2216

Su XD., Guo RH., Yang SY., Kim YH., Kim YR (2019). Anti-bacterial effects of components from Sanguisorba officinalis L. on Vibrio vulnificus and their soluble epoxide hydrolase inhibitory activity. Natural product research. 33(1):3445-3449

Tambo E., Khater EI., Chen J-H., Bergquist R., Zhou X-N (2015). Nobel prize for the artemisinin and ivermectin discoveries: a great boost towards elimination of the global infectious diseases of poverty. BioMed Central. 28(4):58.

Yarahmadi M., Fakhar M., Ebrahimzadeh MA., Chabra A., Rahimi-Esboei B (2016). The anti-giardial effectiveness of fungal and commercial chitosan against Giardia intestinalis cysts in vitro. Journal of parasitic diseases. 40:75-80