Evaluation of Reciprocal Pharmaceutical Effects and Antibacterial Activity of Silver Nanoparticles and Methanolic Extract of *Crocus sativus* L. (Saffron) on Some Bacterial Strains

Anahita Samiee Zafarghandi¹, Hamid Reza Ahmadi Ashtiani², Seyyed Mahdi Rezayat³, Neda Soleimani⁴, Seyyed Reza Hosseinidoost⁵, Shabnam Heydarzadeh Khoyi²

¹MSc Student in Microbiology, Islamic Azad University, Pharmaceutical Sciences Branch, Tehran, Iran
²PhD in Clinical Biochemistry, Islamic Azad University, Pharmaceutical Sciences Branch, Tehran, Iran
³Professor of Pharmacology & Toxicology, Department of Pharmacology, School of Medicine, Islamic Azad University, Pharmaceutical Sciences Branch, Tehran, Iran
⁴Assistant Professor, Department of Microbiology and Microbial Biotechnology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran
⁵Professor of Microbiology, Islamic Azad University, Pharmaceutical Sciences Branch; Tehran, Iran

*Corresponding Author:*
Hamid Reza Ahmadi Ashtiani; PhD in Clinical Biochemistry, Islamic Azad University, Pharmaceutical Sciences Branch, Tehran, Iran
Email: hamidr.ahmadi@yahoo.com

Abstract

**Background:** Silver nanoparticles (Ag-NPs) are the most prominent nanoparticles which are recognized for their high antimicrobial efficacy.

**Objectives:** The aim of this study was to evaluate the reciprocal pharmaceutical effects and antibacterial activity of Ag-NPs and methanolic extract of *Crocus sativus* L. (saffron) on some bacterial strains.

**Materials and Methods:** For evaluation of antibacterial activity of Ag-NPs and methanolic extract of *C. sativus* L. (saffron) on some bacteria, agar well diffusion method was used. Minimal inhibition concentration (MIC) and minimum bactericidal concentration (MBC) were determined for saffron extract, Ag-NPs, and their combination on methicillin-resistant *Staphylococcus aureus* (MRSA) and *S. pyogenes* and *S. epidermidis*.

**Results:** The combination of medium concentrations of Ag-NPs (500 μg/mL) and saffron extract (50 mg/mL) was in the optimum mode to eliminate *S. epidermidis* and *S. pyogenes*. The results showed that saffron extract, Ag-NPs, and their combined form had antibacterial effects on these bacteria.

**Conclusion:** It is suggested to evaluate the synergistic effects of active components of the extract and antimicrobial preservatives used in food, health, pharmaceutical, and cosmetic industries.

Received October 18, 2016; Revised January 11, 2017; Accepted February 10, 2017

Introduction

*Crocus sativus* L., commonly known as saffron, belongs to the family of Iridaceae and is widely cultivated in Iran, India, and Greece.²,³ Saffron is globally the most valuable industrial medical product and Iran is considered as the greatest producer of this plant.⁴ Different parts of saffron including stigma, leaves, stamen and colora represent antimicrobial activity. Traditionally, among these parts stigma was mainly used for its medicinal properties. It also contains a variety of chemical constituents such as crocetin, crocin and other flavonoids, utilized for therapeutic purposes.⁴,⁵ Although antimicrobial activities of saffron extract against various types of microorganisms have already been demonstrated, this research aimed at enhancing this activity. Skin as the largest organ is in direct contact with the environment and is the exposed suitable location for microorganisms. *Staphylococcus*, *Streptococcus* and *Pseudomonas* are the most important bacteria involved in skin diseases.

It is well known that among metal nanoparticles, silver nanoparticles (Ag-NPs) are the most dominant ones. During the past decades, due to unique biological properties of Ag-NPs such as antimicrobial activities, they have been of immense importance.⁷,⁹ Materials at the nanoscale behave different from the same mass of material. Because of higher specific surface area to volume ratio of Ag-NPs compared to bulk silver metal, the resultant antimicrobial effects of Ag-NPs are higher.¹⁰,¹¹ Recently, considering the side effects of antibiotics and occurrence of antibiotic resistance in *Pseudomonas aeruginosa* and *Staphylococcus aureus*, usage of natural agents have attracted the attention of scientists.¹²,¹³ P.
aeruginosa results from low permeability, antibiotic leakage system (efflux system), acquiring resistance genes via plasmids, transposons, and biofilm formation.\textsuperscript{15,16} \textit{P. aeruginosa} is the causative agent of infections in patients with immune deficiency, cystic fibrosis and burns.\textsuperscript{17,18} S. aureus is the most important opportunistic pathogen in hospital infections and is the cause of endocarditis, toxic shock syndrome, furuncle and abscess.\textsuperscript{19-21} Currently, green process is known as an efficient and powerful tool that inspires researchers to avoid usage of toxic chemicals and consequently to protect environment. Specifically, for this purpose herbal extracts can be invaluable resources to synthesize Ag-NPs.\textsuperscript{22-24} In green synthesis protocol, there are no signs of the toxic chemicals, so this process offers numerous benefits including ecofriendliness and compatibility to pharmaceutical applications and also provides advancement due to cost-effectiveness.\textsuperscript{25-28} In this study we reported the enhanced combinatory effects of Ag-NPs and saffron extract against \textit{S. epidermidis}, \textit{S. aureus}, \textit{Streptococcus pyogenes}, \textit{P. aeruginosa}, and methicillin-resistant \textit{S. aureus} (MRSA).

\textbf{Materials and Methods}

\textbf{Preparation of Saffron Extract}

Saffron stigmas that were used in this research were purchased from Novin Saffron Company (Mashhad, Iran). For preparation of methanolic extract of \textit{C. sativus}, the stigmas of plant were dried at room temperature and powdered by electric blender. About 30 g of dry powdered plant material was extracted on the basis of maceration method with methanol (MeOH). In this method, the powdered plant was soaked in methanol for 3 days. The clear solution obtained with the filtering was collected in a glass container and this procedure was repeated 3 times. Finally, the yield from 30 g dry plant was 10 g extraction. Dried extracts were stored in a dark, cool place away from light and moisture.\textsuperscript{14}

\textbf{Preparation of Different Concentrations of Extract}

The prepared methanolic extract was weighted by analytical scale (with accuracy of 0.0001) in the range of 6.25, 12.5, 25, 50, 100 mg. For preparation of the concentrations of 6.25, 12.5, 25, 50, 100 mg/mL, 1 mL of DMSO 10% was added to each of the weighted extracts.

\textbf{Silver Nanoparticles}

Ag-NPs were purchased from Lotus Pasargad Company (Tehran, Iran) in the form of colloidal solution and with the brand of colloidal silver (LNP-CS). This material is completely water soluble and its carrier is distilled water. The concentration of nanoparticles used in this research was 8000 µg/mL and the size of nanoparticles in this product was approximately 40 nm. According to the manufacturers recommendations, monoetilenglicol and distilled water are its solvent and in this study, distilled water was applied as solvent. The concentrations of Ag-NPs colloid were prepared by serial dilution using distilled water.\textsuperscript{15}

\textbf{Preparation of Different Concentrations of Silver Nanoparticles Solution}

To obtain the desired concentrations of Ag-NPs colloid with distilled water, the concentrations of 8000, 4000, 2000, 1000, 500, 250, 125 and 62.5 µg/mL from Ag-NPs in sterile deionized water were prepared with serial dilution.

\textbf{Preparation of Silver Nanoparticles Solution Combined With Saffron Extract}

Minimum, medium and maximum concentrations of saffron extract and nanoparticles, combined\textsuperscript{16} (16) according to the ratio of 1:1, were determined as below (Table 1).

\textbf{Bacterial Strains}

The following 5 strains of bacteria were used for testing, respectively: \textit{S. epidermidis ATCC 12228}, \textit{S. aureus ATCC 25923}, \textit{S. pyogenes ATCC 27853}, \textit{P. aeruginosa ATCC 19615} and MRSA ATCC 3591. It is noteworthy that Microbiology Laboratory of Tarbiat Modares University, Tehran, Iran provided us with these microorganisms.

\textbf{Antimicrobial Assay}

\textit{Agar well diffusion method}, minimal inhibition concentration (MIC) and minimum bactericidal

\begin{table}[h]
\centering
\caption{Combination of Different Concentrations of Saffron Extract and Silver Nanoparticles}
\begin{tabular}{|c|c|c|}
\hline
\textbf{Silver Nanoparticles} & \textbf{Saffron Extract} & \textbf{Number} \\
\hline
Minimum concentration (62.5 µg/mL) & Minimum concentration (12.5 mg/mL) & 1 \\
Minimum concentration (62.5 µg/mL) & Medium concentration (50 mg/mL) & 2 \\
Minimum concentration (62.5 µg/mL) & Maximum concentration (100 mg/mL) & 3 \\
Medium concentration (500 µg/mL) & Minimum concentration (12.5 mg/mL) & 4 \\
Medium concentration (500 µg/mL) & Medium concentration (50 mg/mL) & 5 \\
Medium concentration (500 µg/mL) & Maximum concentration (100 mg/mL) & 6 \\
Maximum concentration (8000 µg/mL) & Minimum concentration (12.5 mg/mL) & 7 \\
Maximum concentration (8000 µg/mL) & Medium concentration (50 mg/mL) & 8 \\
Maximum concentration (8000 µg/mL) & Maximum concentration (100 mg/mL) & 9 \\
\hline
\end{tabular}
\end{table}
concentration (MBC) analyses were the applied three methods to determine the *C. sativus* extract, Ag-NPs solution, and the combinatory antimicrobial activities.

Evaluation of Early Antibacterial Effects of Saffron Extract and Silver Nanoparticles Solution

Antimicrobial activity of the extract was assayed using agar well diffusion method. All the bacteria were incubated at 37°C for 24 hours by inoculation in Mueller-Hinton broth. Each colony was separately suspended in a normal saline solution, so the cultured suspensions were adjusted using 0.5 McFarland turbidity standard tubes. Mueller-Hinton agar was poured into each sterilized Petri dish. The wells each of 6 mm diameter, were made on Petri dishes which were streaked with the microorganism’s saline suspension. Then, 100, 50, 25, 12.5, 6.25 mg/mL of extract and 8000, 4000, 2000, 1000, 500, 250, 125, 62.5 µg/mL of Ag-NPs were prepared and 100 µL of each extract solution was added in wells along with positive control (antibiotic) and negative control (solvent) and Petri dishes were incubated at 37°C for 24 hours. After 24 hours the zone of inhibition produced by each dilution of extract was measured. In this study, 20 mg/mL of Gentamicin was used as positive control for Gram-negative bacteria and 25 mg/mL of vancomycin was used as positive control for gram-positive bacteria. DMSO 10% was used as negative control for methanolic extract and distilled water was used for nanoparticles. In this research the experiments were performed three times and the results were proposed as mean.

Evaluation of Early Antibacterial Effects of Saffron Extract and Silver Nanoparticles Solution Combination

Each colony was separately suspended in a normal saline solution and the cultured suspensions were adjusted using 0.5 MacFarland turbidity standard tubes. Afterwards, 100 µL of each extract solution was added in wells which contained the minimum (12.5 mg/mL), medium (50 mg/mL) and maximum (100 mg/mL) concentrations of methanolic extract and minimum (6.25 µg/mL), medium (500 µg/mL) and maximum (8000 µg/mL) concentrations of nanoparticles solution along with positive control (antibiotic) and negative control (solvent) and Petri dishes that were incubated at 37°C for 24 hours. This procedure also was repeated three times and the average diameter of inhibitory zone of methanolic extract and nanoparticles solution were measured.

Determination of Minimal Inhibition Concentration and Minimum Bactericidal Concentration

In this study, for determination of MIC value, the broth micro dilution method in micro plate was utilized. Based on the evaluation of antibacterial effects of saffron extract, the presence of diameter of inhibitory zone was shown in 2 bacteria of all and we performed MBC value measurement just for these 2 bacteria. But all of the bacteria in this research showed the diameter of inhibitory zone in nanoparticles; so MIC value was measured for all of the bacteria. One hundred microliter of different concentrations of extract, nanoparticles and the combination of extract-nanoparticles were used for each well of micro plate. Then microbial suspension of each strain was prepared according to 0.5 MacFarland in MHB medium and 100 µL of these suspensions were added to each well. The final volume of microbial suspension and final concentrations were calculated by serial dilution method. Each well of micro plate contained medium, bacteria, and extract or nanoparticles or the combination of extract and nanoparticles. Comparing the wells with positive control and negative control can detect the growth or inhibition of bacteria and MIC value. Due to the turbidity in all of the wells, 20 µL of contents of each well was cultured on MHA plates and incubated at 37°C for 24 hours. This procedure was repeated for three times and the colony count was evaluated. The concentration, at which no colonies of bacteria were grown, was reported as MBC. Each test was repeated three times and the results were recorded.

Statistical Analysis

Statistical analysis was performed using SPSS version 15.0 (SPSS Inc., Chicago, IL, USA). For multiple comparisons, data were analyzed by one-way analysis of variance (ANOVA) and followed by LSD test. The *P* value less than 0.05 was considered significant. The results are expressed by mean ± standard deviation (SD).

Results

The antibacterial activity of methanolic extract of saffron was assayed in different concentrations against five species of bacteria by agar well diffusion method. As displayed in Figure 1 and Tables 1 and 2, the saffron extract showed diameters of inhibition zone against *S. epidermidis* and *S. pyogenes* and the mean diameter of inhibition zone regularly increased with higher concentrations of saffron extract. Ag-NPs used in this research had influence on the average diameter of the inhibition zone of *S. aureus*, MRSA, *S. epidermidis*, *S. pyogenes* and *P. aeruginosa*

Figure 1. Average Inhibition Zone Diameter (mm) of *Streptococcus pyogenes* in Different Concentrations of Saffron Extract and Ag-NPs by Agar Well Diffusion Method.
and the mean diameter of inhibition zone increased with higher concentrations of saffron extract. Moreover, combining 2 extracts revealed that the combination cannot influence the mean diameter of inhibition zone but each individually can show this effect. Since higher concentrations of saffron extract and Ag-NPs individually increased, the diameter of inhibition zone when saffron extract and Ag-NPs were in their maximum concentration values, had the highest mean diameter of inhibition zone and vice versa. It means, when saffron extract and Ag-NPs were in their minimum concentration, they had the lowest mean diameter of inhibition zone. According to Tables 1 and 2, various concentrations of saffron extract against S. aureus and S. pyogenes lead to different diameters of inhibition zone and this difference is significant. Moreover, as observed in Tables 1 and 2, an increase in the concentration of saffron extract is directly related with the increase in diameter of inhibition zone.

The most effective MIC values were methanol extracts of C. sativus L. (saffron) for S. epidermidis and (MIC/MBC=50 mg/mL) for S. pyogenes. Remarkably, the methanolic extract containing Ag-NPs exhibited low MIC values in the range of used medium concentration (500 µg/mL) of methanol extract (50 mg/mL), which is very significant in both S. epidermidis and S. pyogenes as expected.

According to the results, effect of different concentrations of Ag-NPs on S. aureus, S. epidermidis, S. pyogenes, MRSA and P. aeruginosa led to different diameters of inhibition zone and this difference was significant. Moreover, as shown in Figures 2-5, with increasing concentration of saffron extract and Ag-NPs, diameter of inhibition zone in abovementioned bacteria raised.

**Discussion**

The current study investigated the influence of different concentrations of C. sativus L. (saffron) against 4 species of gram-positive bacteria and one species of gram-negative bacteria. The inhibitory effects of methanolic extracts of

---

**Table 2. Comparison of Different Concentrations of Saffron Extract in Staphylococcus epidermidis**

| Method          | Bacteria                 | Concentration mg/mL | Extract  | Mean (mm) | Standard deviation (mm) |
|-----------------|--------------------------|---------------------|----------|-----------|-------------------------|
| Agar well       | Staphylococcus epidermidis| 25.0 Van             | Saffron  | 34.5      | 0.58                    |
| diffusion       |                          | 6.25                | Saffron  | -         | -                       |
|                 |                          | 12.5                | Saffron  | 9         | 0                       |
|                 |                          | 25                   | Saffron  | 9.25      | 0.5                     |
|                 |                          | 50                   | Saffron  | 11        | 0.82                    |
|                 |                          | 100                  | Saffron  | 13        | 0.82                    |

*P=0.001.
C. sativus L. were examined on the growth of all species of the tested bacteria. Results showed that S. epidermidis has a greater diameter of inhibition zone in low concentrations and was more sensitive than S. pyogenes. In this research, Ag-NPs showed antibacterial effect on all five species and according to the chart, it seems that S. pyogenes and S. epidermidis were more sensitive than P. aeruginosa. In the study of Im et al.,\textsuperscript{22} it was reported that the antibacterial activity of the Leonuri herbal extract against P. aeruginosa, Escherichia coli and Enterobacter cloacae can be enhanced through the combination with Ag-NPs. Bindhu MR, Umadevi in their study deduced that the prepared Ag-NPs had pronounced antimicrobial activity against Escherichia coli, Proteus mirabilis and Shigella flexneri.\textsuperscript{23} Veerasamy \textit{et al}\textsuperscript{24} suggested that the biosynthesized Ag-NPs using mangosteen leaves extract proved excellent antimicrobial activity. While various theories were proposed to explain the mechanism of antimicrobial activity of Ag-NPs, it is but widely believed that Ag-NPs by penetrating to cell membrane of bacteria and modulating cellular signaling by dephosphorylating putative key-peptide substrates on trypsin residues and tending to bind to sulphur and phosphorous containing compounds like DNA that leads to the condensation of DNA and the loss of its ability to replicate and leakage of intracellular materials can cause the cell death.\textsuperscript{13,14} The other way is explained in terms of the interaction of Ag-NPs with bacterial peptidoglycan cell wall, thus silver ion released from Ag-NPs destroys the peptidoglycan. Moreover, based on recent studies the mechanism mediated by the generation of reactive oxygen species by Ag-NPs may result in the significant antibacterial activity of Ag-NPs.\textsuperscript{14} These studies indicate a simple, reliable, rapid and economical method to synthesize Ag-NPs and furthermore these biologically synthesized nanoparticles are highly effective against multi-drug resistant pathogens.\textsuperscript{29} Dipankar C, Murugan proposed that the biosynthesized Ag-NPs using \textit{Iresine herbstii} leaf aqueous extract represented excellent antimicrobial activity.\textsuperscript{13}

For investigation of the antibacterial activities of methanolic extract-mediated Ag-NPs, the incubation method was performed. Ag-NPs exhibited antibacterial activities against five strains, and \textit{P. aeruginosa} possessed slightly higher MIC/MBC values among the strains. The methanol extract did not show any significant antibacterial activity against \textit{S. aureus}, MRSA and \textit{P. aeruginosa}. It had antibacterial activities only against \textit{S. epidermidis} and \textit{S. pyogenes}. The saffron extract was effective on the average diameter of inhibition zone and this effect was significant. However, the effect of nanoparticles despite their relatively high effectiveness was not significant. Additionally, as expected, the reciprocal effects of combination were not significant. But according to our results, the highest effect on the diameter of inhibitory zone of \textit{S. epidermidis} and \textit{S. pyogenes} was occurred in the highest concentrations of saffron extract. And in \textit{S. pyogenes}, the saffron extract and nanoparticles solution were individually effective on the average diameter of inhibitory zone but the combined form was not effective. It is well known that the high surface per volume ratio and crystallographic surface structure of Ag-NPs result in significant antibacterial activity.\textsuperscript{17} It seems that the methanol extract containing Ag-NPs showed better antibacterial activities against \textit{S. epidermidis} and \textit{S. pyogenes}. However, \textit{S. pyogenes} is more sensitive than \textit{S. epidermidis}.

To conclude, we used low concentrations of nanoparticles for MIC value. The way in which plant extracts and Ag-NPs formulations were used simultaneously to increase the antibacterial activity is very efficient, and can be used for other plants and nanoparticles in the near future. The isolation of different components of saffron extract and evaluation of their antibacterial effects and furthermore evaluation of antiviral and antifungal effects of extract and Ag-NPs are also recommended. Furthermore, it is suggested to evaluate the synergistic effects of active components of the extract and antimicrobial preservatives used in food, health, pharmaceutical and cosmetic industries.

Authors’ Contributions
Study design: AS. Experiment design, experiment conduct, data interpretation and manuscript edition: AS, HRA, NS. Experiment performance: HRA, AS. Data collection, data interpretation, grant application, and manuscript preparation: AS, MR, NS, RH, SHH.

Conflict of Interest Disclosures
The authors declare that they have no conflict of interests.

Ethical Approval
Not applicable.

Financial Support
This study was generously funded by Medical Sciences Research Center, Tehran University, Tehran, Iran.

References
1. Srivastava R, Ahmed H, Dixit RK, Dharamveer, Saraf SA. \textit{Crocus sativus} L. A comprehensive review. Pharmacogn Rev. 2010;4(8):200-208. doi:10.4103/0973-7847.70919.
2. Leffingwell JC. Saffron. Leffingwell reports. 2002;2(5):1-6.
3. Moghaddasi MS. Saffron chemicals and medicine usage. J

Figure 5. Average Inhibition Zone Diameter (mm) of Staphylococcus epidermidis in Different Concentrations of Saffron Extract and Ag-NPs by Agar Well Diffusion Method.
Exp Parasitol. in comparison L. (saffron). Int J Pharm Pharm Sci. leaf extracts and its. Nanomedicine. 2007;3(2):168-171. on J Med Microbiol. 1988;25:109-114. Surveill of with serotyping and pyocin typing for epidemiological Poh CL, Yap EH, Tay L, Bergan T. Plasmid profiles compared infection control study. Burns. 2001;27:131-135. Resistant Int J Nanomedicine. 2012;7:4263-4267. Effect of silver nanoparticles-chitosan composite particles. Based Complement Alternat Med. 2009;6(3):343-350. Activity of silver nanoparticles on ischemia-reperfusion in rat skeletal muscle. Evid L. (Saffron) extract and its active constituents (crocin and Hosseinzadeh H, Modaghegh MH, Saffari Z. Spectrum B-lactamase enzymes. Medical Laboratory Journal. 2011;3(2):45-51. Hosseinazedeh H, Mogadegh MH, Safaari Z. Crocus sativus L. (Saffron) extract and its active constituents (crocin and safraanal) on ischemia-reperfusion in rat skeletal muscle. Evid Based Complement Alternat Med. 2009;6(3):343-350. Wang LS, Wang CY, Yang CH, et al. Synthesis and anti-fungal effect of silver nanoparticles-chitosan composite particles. Int J Nanomedicine. 2015;10:2685-2696. doi:10.2147/IJN. L77410. Loo YY, Chieng BW, Nishibuchi M, Bao S. Synthesis of silver nanoparticles by using tea leaf extract from Camellia sinensis. Int J Nanomedicine. 2012;7:4261-4267. Douglas WM, Mulholand K, Denny V, Gottlieb T. Multi-drug resistant Pseudomonas aeruginosa outbreak in a burns unit an infection control study. Burns. 2001;27:131-135. Poh CL, Yap EH, Tay L, Bergan T. Plasmid profiles compared with serotyping and pyocin typing for epidemiological surveillance of Pseudomonas aeruginosa. J Med Microbiol. 1988;25:109-114. 19. Soleimani N, Sattari M, Sepehrisaresht S, Daneshmandi S. Evaluation of reciprocal pharmaceutical effects and antibacterial activity of Banium persicum essential oil against some Gram positive and Gram negative bacteria. Iran J Med Microbiol. 2010;4(1-2):26-34. 20. Shopsin B, Kreiswirth B. Molecular epidemiology of methisillin-resistant Staphylococcus aureus. Emerg Infect Dis. 2001;7(2):323-326. 21. Stegner VE, Weiss AA. Preliminary study of methodologies for detection of methicillin-resistant Staphylococcus aureus. Lab Med. 2013;44(1):74-78. doi:10.1309/ LMC6B0TWUC4DMQQP. 22. Im AR, Han L, Kim ER, Kim J, Kim YS, Park Y.. Enhanced antibacterial activities of leonuri herba extracts containing silver nanoparticles. Phytother Res. 2012;26(8):1249-1255. 23. Prashanth S, Menaka I, Muthezhilkan R, Sharma NK. Synthesis of plant-mediated silver nano particles using medicinal plant extract and evaluation of its anti microbial activities. Int J Eng Sci Thecnol. 2011;3(8):6235-6250. 24. Daneshmandi S, Soleimani N, Pourtahothallah AA, Sattari M. Evaluation of the drug synergystic and antibacterial effects of cuminum cyminum essential oil (Persian). Arak Medical University Journal. 2010;3(2):75-82. 25. Santhoshkumar T, Rahuman AA, Bagavan A, et al. Evaluation of steam aqueous extract and synthesized silver nanoparticles using Cissus guadangulata against Hippobosca maculate and Rhipephalus (Boophilus) microplus. Exp Parasitol. 2012;132(2):156-165. doi:10.1016/j.exppara.2012.06.009. 26. Dubey SP, Lahitnen M, Sillanpää M. Green synthesis and characterization of silver and gold nanoparticles using leaf extract of Rosa rugosa. Colloids Surf A Physicochem Eng Asp. 2010;364:34-41. doi:10.1016/j.colsurfa.2010.04.023. 27. Haghhi F, Roudbar Mohammadi S, Soleimani N, Sattari M. Evaluation of anti fungal activity of essential oils of Thymus vulgaris, Petroselium Crispum, Cuminum cyminum and Banium persicum on Candida albicans in comparison with Fluconazole. Modares Journal of Medical Sciences: Pathobiology. 2011;14(1):29-35. 28. Smitha SL, Philip D, Gopchandran KG. Green synthesis of gold nanoparticles using Cinnamomum zeylanicum leaf broth. Spectrochim Acta A Mol Biomol Spectrosc. 2009;74(3):735-9. doi:10.1016/j.saa.2009.08.007. 29. Bindhu MR, Umadevi M. Synthesis of monodispersed silver nanoparticles using Hibiscus cannabinus leaf extract and its antimicrobial activity. Spectrochim Acta A Mol Biomol Spectrosc. 2013;101:184-90. doi:10.1016/j.saa.2012.09.031. 30. Veerasamy R, Xin TZ, Gunasagar S, et al. Biosynthesis of silver nanoparticles using mangosteen leaf extract and evaluation of their antimicrobial activities. J Saudi Chem Soc. 2011;15(1):113-120. doi:10.1016/j.jscs.2010.06.004. 31. Dipankar C, Murugan S. The green characterization and evaluation of the biological activities of silver nanoparticles synthesized from Iresine herbstii leaf aqueous extracts. Colloids Surf B Bioiinterfaces. 2012;98:112-119.