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Anti-biofilm, Antioxidant and Haemolysis Effect of Silver, Chitosan and Curcumin Nanoparticles on Antibiotics Resistance *Klebsiella pneumonia*Isolates From Urinary Tract Infection

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Abstract
This study was completed in laboratories of Biology Department in Faculty of Science. It explains the anti-biofilm, antioxidant and haemolysis effect ofsilver, chitosan and curcumin nanoparticles on antibiotics resistance *Klebsiella pneumonia* that isolated from urinary tract infection patients in the province of Thi Qar.A total number of (200) samples were collected from patients with urinary tract infectionfrom AL-Hussein Teaching Hospital and AL-Shatrah General Hospital in Thi-Qar Governorate, the capacity of some *K. pneumonia* isolates to biofilm formation was detected by phenotypic method which included Congo Red Agar method (CRA), from the 65 (100%) isolates of *K. pneumonia* 58 (89.23 %) were biofilm producers when that appearance of black dry crystalline colonies on the CRA plates and 7 (10.77 %) were non-biofilm producers when the colonies of *K. pneumonia*remained pink or red colored. The results showed that sliver, chitosan and curcuminNPsexpressed high anti-biofilm activity via plate method against *K. pneumoniae* isolateswith increasing concentrations of silver, chitosan and curcuminNPs (100, 200 ,300 and 400) µg/ml.Silver, chitosan and curcumin nanoparticles with four concentrations of (100, 200, 300, 400) µg/ml showed antioxidant activity using DPPH methods. DPPH reducing activity of nanoparticles increased with the increase in the concentration of nanoparticles, also DPPH reducing activity of nanoparticles increased with the mix (silver, chitosan and curcumin) NPs compared with the presence of silver, chitosan and curcumin nanoparticles alone. It was 48%, 54%, 57%, and 59% in (100, 200, 300 and 400 µg/ml) respectively for silver NPs, also, 39%, 42%, 46%, and 48% in (100, 200, 300 and 400 µg/ml) respectively for chitosan NPs, and 42%, 48%, 53%, and 55% in (100, 200, 300 and 400 µg/ml) respectively for curcumin NPs. It was 52%, 63%, 71% and 80% in (100, 200, 300 and 400 µg/ml) respectively for the mix (silver, chitosan and curcumin) NPs. Silver, chitosan and curcumin nanoparticles with all concentration (100, 200, 300, 400) µg/ml also, the mix (silver, chitosan and curcumin) NPs together did not show any hemolysis for the tested whole blood.

Keywords: Anti-biofilm, Antioxidantactivity,Haemolysis,Nanoparticles,

1.Introduction

Urinary tract infection is one of the widespread diseases, as well as it is characterized by multiple causes of bacterial pathogens and both sexes and all ages (1).UTIs are categorized as uncomplicated or complicated. Uncomplicated UTIs typically affect individuals who are otherwise healthy and have no structural or neurological urinary tract abnormalities, these infections are differentiated into lower UTIs (cystitis) and upper UTIs (pyelonephritis), several risk factors are associated with cystitis, including female gender, a prior UTI, sexual activity, vaginal infection, diabetes, obesity and genetic susceptibility (2). Complicated UTIs are defined as UTIs associated with factors that compromise the urinary tract or host defense, including urinary obstruction, urinary retention caused by neurological disease, immunosuppression, renal failure, renal transplantation, pregnancy and the presence of foreign bodies such as calculi, indwelling catheters or other drainage devices (3). Antibiotic resistance was informed to occur when a drug loses its capability to inhibit bacterial growing efficiently, bacteria change resistant' and remain 'to grow in the occurrence of therapeutic levels of the antibiotics, bacteria , when duplicates even in the presence of the antibiotics are called resistant, bacteria, antibiotics become are typically active against them, but when the microbes fewer sensitive or

resistant, it needs a greater than the normal concentration of the identical drug to have an influence (4).

2. Materials and Methods

2.1:Samples collection and bacterial identification

A total number of 200 (urine samples) were collected from patients with urinary tract infection (UTI) from AL-Shatrah General Hospital and AL-Hussein Teaching Hospital in Thi-Qar Governorate during the period from (January, 2022 to April, 2022).The mid-stream urine specimens were collected from patients in sterilized screw-cap container and transportedto advanced Microbiology laboratoryof Science College in Kufa University and culturing on diverse media for 24 hours at cultivate 37°C for bacterial diagnosis.

2.2: Silver, chitosan and curcumin NPs as anti-biofilm, antioxidantand haemolysis agent

2.2.1: Preparation of silver, chitosan and curcumin nanoparticles: according of (5), (6) and (7)respectively.

2.2.2: Preparation of Congo Red Agar: according of (8)

2.3:Detection of biofilm formation for *Klebsiella pneumonia*e isolates

Biofilm production by isolated from UTI pathogens in our study was detected by phenotypic method which included Congo Red Agar Method (CRA), prepared CRA plates were inoculated with the *Klebsiellapneumonia*e isolates and aerobically incubated at 37°C for 48 h, appearance of black dry crystalline colonies on the CRA plates indicated positive biofilm production while the colonies of biofilm no producer remained pink or red colored negative (9).

2.4:Anti-biofilm formation of the silver NPs against *K.pneumoniae* isolates

Microtiterplate method was used for in vitro anti-biofilm activity, four concentrations 100, 200, 300 and 400 µg/ml of silver NPs, 0.1ml of cell suspension having 0.5 O.D at 630 nm have been inoculated in 1.9 ml BHIB medium, 150ul of the cultured BHIB then transferred into each well of 96-well microtiter plate in use, an amount of 50ul of each 4X concentration was added to the corresponding wells to obtain the final concentrations, an amount of 50ul of BHIB was added one well corresponding to *K. pneumoniae* isolate used as control to confirm production of biofilm by bacteria and inhibition of biofilm formation by silver NPs, an amount of 200µl of autoclaved distilled water was added in peripheral wells (to reduce the water loss), microtiter plate was incubated for 16 h at 37°C, planktonic cells then aspirated, and fixed with 99% methanol, plates then washed twice with phosphate buffer saline or sterile saline water and air-dried, about 200µl of crystal violet solution (0.2%) then added to all wells, after 5 min, excess crystal violet was removed and washed twice, after that the plate was air dried and the cell bound crystal violet was dissolved in 33% acetic acid, the optical density (O.D.) at 630nm was recorded (10).

2.5:Anti-biofilm formation of the chitosan and curcumin NPs against *K. pneumonia* isolates

This test is agreed in the same manner described in paragraph (2.4**)** excluding the use of chitosan and curcumin NPs.

2.6:Anti-biofilm formation of mix the NPs against *K. pneumonia* isolates

This test is agreed in the same manner described in paragraph (2.4**)** excluding the use of mix the (silver, chitosan and curcumin) NPs.

2.7:Antioxidant activity of silver, chitosan and curcumin NPs

DPPH was used to determine the extracts free radical scavenging capability, the DPPH solution (0.006 % w/v) was prepared in 95% methanol, freshly prepared DPPH solution was placed in test tubes, and NPs (100, 200, 300, 400 ug/ml) were applied to each test tube until the final volume was 2 ml, and discoloration was calculated at 517 nm (UV visible spectrophotometer) after 30 minutes in the dark incubation.DPPH solution was used as a control, and 95% methanol was utilized as a blank. Percentage of DPPH free radical scavenging was calculated using the following equation:

DPPH scavenging impact (%) = (Ao –A1) / Ao× 100, where Ao was the absorbance of the control and A1 was the absorbance in the presence of the nanoparticles (11).

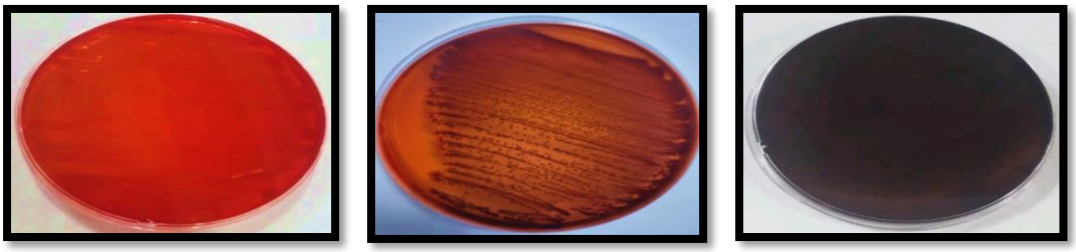
2.8:Haemolysis effect of silver, chitosan and curcumin NPs

Thehaemolytic toxicity of silver, chitosan and curcumin NPs was calculated by measuring the percentage of haemolysis, blood was taken from a normal un medicated human donor and collected in anticoagulant EDTA, 2 mL blood was applied to each tube with NPs concentrations of (100, 200, 300, 400 µg/ml), 2 mL blood was immediately drawn for initial examination, and the remaining 2 mL blood was incubated for 30 minutes with NPs (12).Triton X-100 1% was used as indicator of positive control. In each sample, the amount of free Hb released into the plasma following exposure to the samples was measured (13).The percentage of haemolysis was determined using the formula: percent Haemolysis = Free Hb / Total Hb 100.

3.Results and Discussion

3.1: The ability of *K. pneumonia*e isolates to form biofilm

The results showed that the capacity of some *K. pneumonia*isolates to biofilm formation was detected by phenotypic method which included Congo Red Agar Method (CRA), from the 65 (100%) isolates of *K. pneumonia* 58 (89.23 %) were biofilm producers when that appearance of black dry crystalline colonies on the CRA plates and 7 (10.77 %) were non - biofilm producers when the colonies of *K. pneumonia*remained pink or red colored (Figure 1).



A **B** **C**

Figure 1: Biofilm formation by some*K. pneumonia*isolatesA: without Biofilm formation B &C: Biofilm formation

3-2: Anti-biofilm activity of different concentrations of sliver, chitosan and curcumin NPs of MDR *K. pneumoniae* isolates

The results showed that sliver, chitosan and curcuminNPsexpressed high anti-biofilm activity via plate method against *K. pneumoniae* isolateswith increasing concentrations of silver, chitosan and curcuminNPs (100, 200 ,300 and 400) µg/ml(Table 1-4).

Table 1: Anti-biofilm activity of silver NPs at four concentrations gainst *K. pneumoniae*isolates

No. of *K. pneumoniae*isolates	Absorbance of silver NPs (µg/ml)			
	100	**200**	**300**	**400**
K.P 1	0.398	0.274	0.179	0.080
K.P 2	0.265	0.213	0.158	0.069
K.P 3	0.297	0.258	0.159	0.030
K.P 4	0.395	0.328	0.152	0.048
K.P 5	0.369	0.286	0.133	0.082
K.P 6	0.336	0.273	0.113	0.092
K.P 7	0.299	0.256	0.124	0.082
K.P 8	0.373	0.297	0.169	0.070
K.P 9	0.394	0.267	0.178	0.069
K.P 10	0.374	0.299	0.142	0.029
K.P 11	0.319	0.255	0.139	0.044
K.P 12	0.395	0.317	0.130	0.046
K.P 13	0.288	0.230	0.124	0.057
K.P 14	0.325	0.300	0.135	0.072
K.P 15	0.282	0.251	0.118	0.067

K.P 16	0.342	0.271	0.199	0.086
K.P 17	0.367	0.328	0.145	0.034
K.P 18	0.289	0.219	0.127	0.044
K.P 19	0.317	0.293	0.126	0.089
K.P 20	0.280	0.229	0.118	0.064
K.P 21	0.380	0.249	0.128	0.100
K.P 22	0.306	0.289	0.136	0.070
K.P 23	0.394	0.319	0.185	0.079
K.P 24	0.299	0.219	0.154	0.014
K.P 25	0.350	0.215	0.192	0.038
K.P 26	0.377	0.242	0.110	0.086
K.P 27	0.349	0.221	0.109	0.081
K.P 28	0.299	0.215	0.143	0.012
K.P 29	0.314	0.286	0.163	0.036
K.P 30	0.359	0.218	0.173	0.042
K.P 31	0.296	0.258	0.129	0.084
K.P 32	0.375	0.297	0.169	0.073
K.P 33	0.397	0.269	0.174	0.062
K.P 34	0.370	0.295	0.140	0.025
K.P 35	0.319	0.258	0.135	0.045
K.P 36	0.399	0.317	0.133	0.041
K.P 37	0.288	0.234	0.127	0.050
K.P 38	0.328	0.303	0.133	0.079
K.P 39	0.285	0.254	0.110	0.067
K.P 40	0.348	0.270	0.194	0.081

Table 2: Anti-biofilm activity of chitosan NPs at four concentrations against *K. pneumoniae*isolates

No. of *K. pneumoniae*isolates	**Absorbance of chitosan NPs (µg/ml)**			
	100	**200**	**300**	**400**
K.P 1	0.397	0.270	0.177	0.088
K.P 2	0.246	0.219	0.154	0.062
K.P 3	0.291	0.256	0.153	0.037
K.P 4	0.386	0.329	0.155	0.043
K.P 5	0.369	0.283	0.130	0.089
K.P 6	0.339	0.272	0.118	0.092
K.P 7	0.299	0.257	0.123	0.081
K.P 8	0.379	0.298	0.166	0.070
K.P 9	0.394	0.269	0.175	0.068
K.P 10	0.374	0.298	0.142	0.021
K.P 11	0.319	0.255	0.133	0.048
K.P 12	0.390	0.316	0.139	0.044
K.P 13	0.289	0.237	0.124	0.050
K.P 14	0.327	0.309	0.134	0.079
K.P 15	0.282	0.250	0.117	0.066
K.P 16	0.342	0.271	0.199	0.084

K.P 17	0.367	0.329	0.143	0.038
K.P 18	0.280	0.218	0.122	0.039
K.P 19	0.313	0.229	0.160	0.087
K.P 20	0.288	0.213	0.110	0.066
K.P 21	0.284	0.248	0.115	0.028
K.P 22	0.301	0.287	0.135	0.077
K.P 23	0.349	0.310	0.183	0.079
K.P 24	0.399	0.277	0.152	0.019
K.P 25	0.357	0.319	0.193	0.031
K.P 26	0.279	0.216	0.118	0.084
K.P 27	0.343	0.229	0.105	0.081
K.P 28	0.298	0.200	0.142	0.011
K.P 29	0.310	0.284	0.169	0.035
K.P 30	0.358	0.319	0.177	0.041
K.P 31	0.369	0.289	0.133	0.088
K.P 32	0.338	0.273	0.112	0.099
K.P 33	0.297	0.219	0.129	0.083
K.P 34	0.378	0.299	0.166	0.078
K.P 35	0.399	0.325	0.178	0.064
K.P 36	0.377	0.299	0.146	0.028
K.P 37	0.314	0.258	0.137	0.046
K.P 38	0.391	0.315	0.138	0.047
K.P 39	0.284	0.236	0.124	0.055
K.P 40	0.323	0.308	0.139	0.073

Table 3: Anti-biofilm activity of Curcumin NPs at four concentrations against *K. pneumoniae*isolates

No. of *K. pneumoniae*isolates	**Absorbance of Curcumin NPs (µg/ml)**			
	100	**200**	**300**	**400**
K.P 1	0.386	0.288	0.198	0.067
K.P 2	0.299	0.231	0.151	0.095
K.P 3	0.292	0.264	0.198	0.061
K.P 4	0.362	0.333	0.198	0.042
K.P 5	0.383	0.252	0.218	0.045
K.P 6	0.379	0.297	0.107	0.088
K.P 7	0.258	0.211	0.196	0.077
K.P 8	0.379	0.278	0.155	0.087
K.P 9	0.386	0.302	0.185	0.084
K.P 10	0.387	0.281	0.155	0.093
K.P 11	0.319	0.257	0.138	0.049
K.P 12	0.399	0.316	0.139	0.048
K.P 13	0.286	0.239	0.125	0.053
K.P 14	0.322	0.308	0.131	0.072

K.P 15	0.289	0.258	0.116	0.068
K.P 16	0.347	0.270	0.195	0.086
K.P 17	0.365	0.328	0.149	0.091
K.P 18	0.288	0.216	0.129	0.033
K.P 19	0.318	0.297	0.169	0.085
K.P 20	0.289	0.200	0.115	0.066
K.P 21	0.289	0.247	0.118	0.045
K.P 22	0.308	0.287	0.136	0.075
K.P 23	0.349	0.317	0.186	0.079
K.P 24	0.290	0.277	0.158	0.016
K.P 25	0.355	0.318	0.295	0.039
K.P 26	0.274	0.218	0.110	0.087
K.P 27	0.340	0.327	0.206	0.109
K.P 28	0.297	0.220	0.146	0.016
K.P 29	0.318	0.288	0.165	0.100
K.P 30	0.358	0.319	0.274	0.116
K.P 31	0.297	0.259	0.126	0.089
K.P 32	0.377	0.299	0.164	0.075
K.P 33	0.393	0.368	0.172	0.061
K.P 34	0.379	0.299	0.144	0.022
K.P 35	0.316	0.256	0.130	0.043
K.P 36	0.392	0.311	0.139	0.040
K.P 37	0.285	0.238	0.126	0.055
K.P 38	0.323	0.308	0.134	0.079
K.P 39	0.282	0.259	0.110	0.066
K.P 40	0.349	0.272	0.199	0.081

3-3: Antioxidant Activity of Silver, Chitosan and Curcumin NPs

The results revealed the ability of NPs to scavenge DPPH free radicals, indicated by observing the colour change, DPPH reducing activity of nanoparticles increased with the increase in the concentration of biogenic nanoparticles, also DPPH reducing activity of nanoparticles increased with the mix (silver, chitosan and curcumin) NPs compared with the presence of silver, chitosan and curcumin nanoparticles alone. It was 48%, 54%, 57%, and 59% in (100, 200, 300 and 400 µg/ml) respectively for silver NPs, also, 39%, 42%, 46%, and 48% in (100, 200, 300 and 400 µg/ml) respectively for chitosan NPs, and 42%, 48%, 53%, and 55% in (100, 200, 300 and 400 µg/ml) respectively for curcumin NPs. It was 52%, 63%, 71% and 80% in (100, 200, 300 and 400 µg/ml) respectively for the mix (silver, chitosan and curcumin) NPs (Figures 2-4).

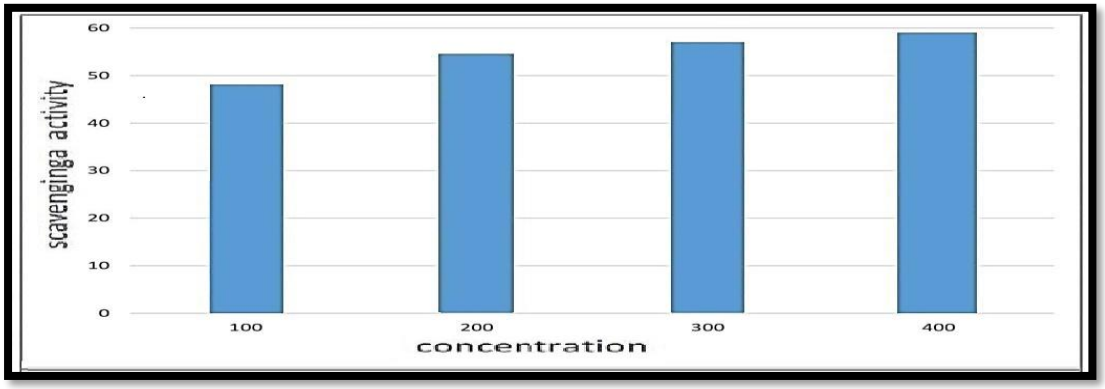


Figure 2: Antioxidant activity of silver NPs by DPPH assay

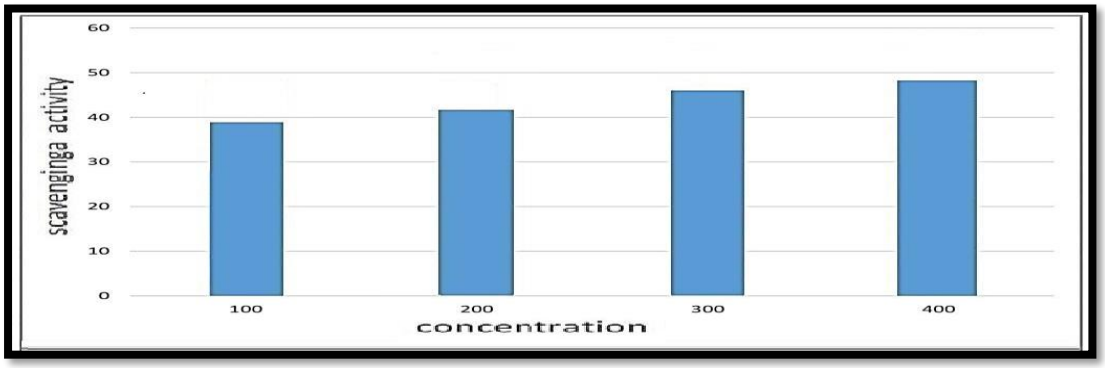


Figure 3: Antioxidant activity of chitosan NPs by DPPH assay

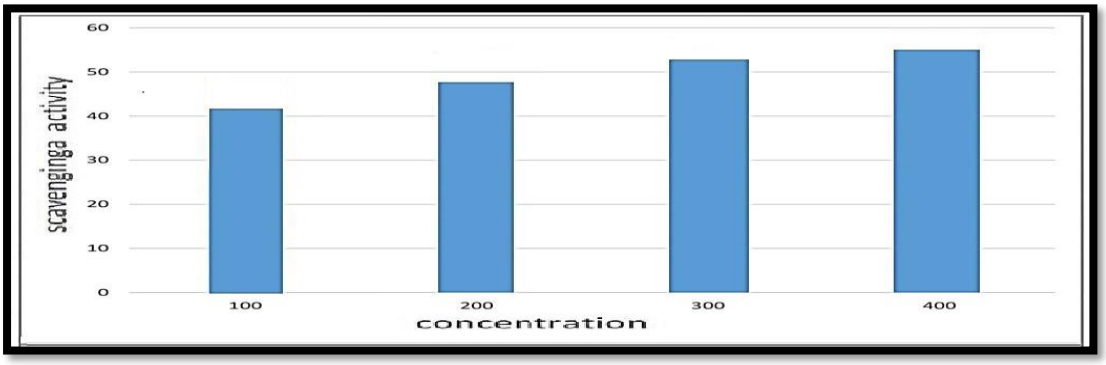


Figure 4: Antioxidant activity of curcumin NPs by DPPH assay

3-4: Haemolysis activity of nanoparticles

Haemolysis was detected by using Triton X-100 as indicators of positive control, sterile solution of phosphate buffer saline was used as a negative control that could store the stock solution at room temperature, Silver, chitosan and curcumin NPs with all concentration (100, 200, 300 and 400 µg/ml) also, the mix (silver, chitosan and curcumin) NPs together did not show any haemolysis for the tested whole blood table (3-4).

Table 4: Haemolysis activity of nanoparticles

Sample	Haemolysis %
Triton X-100 (positive control)	100
PBS (negative control)	0
Blood with Silver NPs 100, 200, 300, 400 µg/ml	0
Blood with Chitosan NPs 100, 200, 300, 400 µg/ml	0
Blood with Curcumin NPs 100, 200, 300, 400 µg/ml	0
Blood with (Silver, Chitosan and Curcumin) NPs 100, 200, 300, 400 µg/ml	0

REFERENCES

1. Bono, M. J. and Reygaert, W. C. (2022). Urinary Tract Infection. In: Stat Pearls [Internet]. Treasure Island (FL): Stat Pearls Publishing.
2. Fazly Bazzaz, B.S., Darvishi Fork, S., Ahmadi, R. et al. (2021). Deep insights into urinary tract infections and effective natural remedies. Afr J Urol 27, 6.
3. Sabih, A., Leslie, S.W. (2022). Complicated Urinary Tract Infections. [Updated 2021 Aug 12]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing.
4. Rahman, AE., Iqbal, A., Hoque DE, et al. (2017). Managing neonatal and early childhood syndromic sepsis in sub-district hospitals in resource poor settings: improvement in quality of care through introduction of a package of interventions in rural Bangladesh. PloS one. , 12:10.1371.

5. Devi, J.S., Bhimba, B.V., and Ratnam, K. (2012). In vitro anticancer activity of silver nanoparticles synthesized using the extract of Gelidiella sp. Int. J. Pharm. Pharm. Sci., pp. 710-715.
6. Madureira, A.R., Pereira, A., Castro, P.M., and Pintado, M. (2015). Production of antimicrobial chitosan nanoparticles against food pathogens, J. Food Eng. 167 (Part B) 210–216.
7. Artur, Adamczak, Marcin O ˙zarowski and Tomasz M. Karpi ´nski. (2020). Curcumin a Natural Antimicrobial Agent with Strain-Specific Activity. Pharmaceuticals 13, 153.
8. Hassan, A., Usman, J., Kaleem, F., Omair, M., Khalid, A. and Iqbal, M. (2011). Evaluation of different detection methods of biofilm formation in the clinical isolates. Braz J Infect Dis;15:305-11.
9. Ruchi, T., Sujata, B., and Anuradha, D. (2015). Comparison of phenotypic methods for the detection of biofilm production in uro-pathogens in a tertiary care hospital in India. Int J Curr Microbiol App Sci, 4(9), 840–849.
10. Shukla, S. K., and Rao, T. S. (2017). An Improved Crystal Violet Assay for Biofilm Quantification in 96-Well Microtitre Plate. BioRxiv, 100214.
11. Goyal, A. K., Middha, S. K., and Sen, A. (2010). Evaluation of the DPPH radical scavenging activity, total phenols and antioxidant activities in Indian wild Bambusa vulgaris" Vittata" methanolic leaf extract. Journal of Natural Pharmaceuticals, 1(1).
12. Dey, S., Sherly, M. C. D., Rekha, M. R., and Sreenivasan, K. (2016). Alginate stabilized gold nanoparticle as multidrug carrier: Evaluation of cellular interactions and hemolytic potential. Carbohydrate Polymers, 136, 71–80.
13. Mesdaghinia, A., Pourpak, Z., Naddafi, K., Nodehi, R. N., Alizadeh, Z., Rezaei, S., Mohammadi, A., and Faraji, M. (2019). An in vitro method to evaluate hemolysis of human red blood cells (RBCs) treated by airborne particulate matter (PM 10). MethodsX, 6, 156–161.