ANTIMICROBIAL ACTIVITY OF SELECTED SPICE EXTRACTS ON *ESCHERICHIA COLI*, *SALMONELLA SPP*., AND *LISTERIA MONOCYTOGENES*

ORIGINAL SCIENTIFIC PAPER

Vildana Jogić[1]✉, Aida Džaferović[1], Merima Toromanović[1], Jelena Nikitović[2]

DOI: 10.51558/2232-7568.2023.16.1.23

RECEIVED 2023-03-31 ACCEPTED 2023-06-27

[1] Biotechnical Faculty, University of Bihać, Luke Marjanovića bb, Bihać, Bosnia and Herzegovina
[2] Institute of Banja Luka, Bosnia and Herzegovina, Bulevar vojvode Petra Bojovića 1A, Banja Luka, Bosnia and Herzegovina

✉ vildana.ahmed@gmail.com

ABSTRACT:
The study determined antimicrobial activity using the Disc-diffusion method and extracts of three plants: garlic (*Allium sativum* L.), turmeric (*Curcuma longa* L.), and parsley (*Petroselinum crispum* L.) on *Escherichia coli*, *Salmonella ssp*. and *Listeria monocytogenes*. These pathogens have attracted the attention of numerous agencies and researchers, because of the negative impact on food and human health - and because of the impact of disease development on the economy. These studies are necessary and represent potential natural antimicrobial drugs, and due to the resistance of bacteria to known antibiotics, there is a constant increase in global consumer demand for natural ingredients. The tested plant extracts showed excellent antibacterial activity in all three replicates on *Escherichia coli* with an average inhibition zone of 21,86 mm and were characterized as ***S - sensitive for the mentioned bacterium. With an average zone of inhibition of 19,052 mm, *Curcuma longa* showed the strongest effect on the tested bacterium *Salmonella enteritidis*, which tells us that this bacteria is very sensitive to the extract of the mentioned plant. The study identified zones of inhibition of very low values (> 8 mm) in all three tested extracts: *Allium sativum* L., *Curcuma longa* L. *Petroselinum crispum* L., and *Listeria monocytogenes*.

KEYWORDS: Antimicrobial activity, extract, Disc-diffusion method

INTRODUCTION

One of the leading problems of the food industry is the microbiological spoilage of food and food poisoning, and as a result, various diseases are appearing. According to (Anand and Sati [1] food preservation is the main concern of the food industry today due to the existing preservatives that are based on synthetic chemicals, but they also show a negative effect on human health. Spices are mainly of plant origin, with a specific smell and taste, they are added to food or food products and drinks in order to achieve the appropriate smell, taste and color, but they also exhibit significant antioxidant and antimicrobial activity, which is why they are increasingly used as natural preservatives [2]. They can be consumed fresh, dried, chopped, turned into powder and as extracts of aromatic ingredients [3]. Due to the presence of the mentioned substances, they also show an effect on the digestive tract, and achieve the secretion of saliva, that is, the secretion of the glands, and thus a greater secretion of digestive enzymes, thus improving digestion.

Research carried out by Melvin et al. [4] the antimicrobial and antifungal activity of spices is also based on the presence of essential oils that contain sulfur and alkaloids, and it is very important to use fresh spices in the daily diet, as an addition to meals, since older spices, in addition to being poorer in essential oils, can cause some side effects after use. Due to their important properties, spices have become indispensable for culinary world-wide, and spice trade has been an important commercial activity since ancient times and a means of economic development [5]. Between the year 2000. and 2019., the value of spice imports increased by 2,9% per year, and the volume increased by 5,9%. In 2019, the market of spices was about 1,547 million tons worth 2,97 billion US dollars, which reflects the importance of spices in the world and their demand [6].

The main goal of the conducted experimental research is to determine the antimicrobial activity of extracts of three selected types of herbs: garlic (*Allium sativum* L.), turmeric (*Curcuma longa* L.) and parsley (*Petroselinum crispum* L.) against the following bacteria: *Escherchia coli*, *Salmonella ssp*., *Listeria* *monocytogenes*. The assumption is that the antimicrobial activity of the mentioned extracts will have a negative effect on the survival ability of pathogenic strains of *Salmonella ssp*., *Listeria monocytogenes* and *Escherchia coli* in foods, because the extracts from spices, in addition to providing meat and dairy products with a characteristic taste, also show an antimicrobial effect and in this way they prevent the development of bacteria, and thus the process of food spoilage.

MATERIAL AND METHODS

For the conducted research, methanol extracts of herbs were used as samples: Parsley - *Petroselinum crispum*/leaf; Garlic - *Allium sativum* L./bulb and Turmeric - *Curcuma longa*/tuber-powder, and the bacterial isolates used are: *Escherichia coli* ATCC 25922, *Salmonella enteritidis* ATOC 13076 and *Listeria monocytogenes* ATCC 19115. Herbs parsley (*Petroselinum crispum* L.) and garlic (*Allium sativum* L.) were homogenized and chopped, and turmeric powder per 50 g sample was extracted with 100 ml of 80% methanol for 24 h at room temperature. Extracts of these spices were obtained by filtering on a Bihners funnel. After filtering the extracts, methanol was separated using a rotary evaporator (Rotavapor R-210/215 BÜCHI, Buchi AG, Flawil, Switzerland) at 40°C. The extracts prepared in this way represent the samples used to determine the antimicrobial activity.

Isolation of Salmonella enteritidis - after preparating the dilution of the sample (sample positive for *Salmonella ssp*. from the Veterinary Institute of USK), isolation of *Salmonella enteritidis* ATOC 13076 was carried out according to the directives BAS EN ISO 6579:2005, BAS EN ISO 6579/cor1 (2010).

Additional identification was done by sowing isolated bacteria on XLD agar (xylose lysine deoxycholate agar; Torlak, Belgrade, Serbia) on a non-selective medium. Isolated Salmonella enteritidis ATOC 13076 was seeded on Salmonella Chromogenic Agar (Torlak, Belgrade, Serbia). Additional identification was done by sowing isolated bacteria on XLD agar (xylose lysine deoxycholate agar; Torlak, Belgrade, Serbia) on a non-selective medium. Isolated *Salmonella enteritidis* ATOC 13076 was seeded on *Salmonella Chromogenic* Agar (Torlak, Belgrade, Serbia).

Isolation of *Listeria monocytogenes* - detection of *Listeria monocytogenes* was performed using the Horizontal method BAS EN ISO 11290-1:2018 (sample positive for Listeria monocytogenes) from the Veterinary Institute of the USK. Detection of *L. monocytogenes* takes place successively, by primary and secondary enrichment, and by seeding on selective substrates Aloe and Oxford agar. Typical colonies on Aloe agar are green-blue surrounded by an opaque halo, while on Oxford agar typical colonies are small (1 mm) and grayish colonies with a black halo, which after 48 h become darker with a greenish sheen and have black halos and a sunken center. Colonies suspected of being L. monocytogenes go for further confirmation, which includes catalase test, Gram stain, hemolysis test, acid formation by decomposition of carbohydrates and Camp test **.**

Isolation of *Escherichia coli* - by the Membrane Filtration method - a very popular test in microbiology of water. The presence and number of *Escherichia coli* can be determined by using membrane filtrates. The selected volume of water is filtered (water sample suspected of *E.coli*), and any bacteria present remain on the filter. The filter is then removed from the funnel, placed on a solid selective medium in a petri dish (most often EMB or endoagar), and then incubated at 35 to 37°C or at 44,5°C. Colonies of *E.coli* present on the filter will grow within 24 to 48 hours.

The disc-diffusion method is the most frequently used qualitative method for determining resistance in microorganisms. It is performed by applying an inoculum containing approximately 1 to 2 × 108 log CFU/mL of the tested microorganism to the surface of a Petri plate with a suitable nutrient medium, and then paper discs impregnated with a fixed concentration of antibiotic and sample are placed on it. The results are read after 16 of 24 h, by measuring the growth inhibition zone of the tested microorganisms [7]. Although the disk-diffusion method is most often used with clinical isolates, because it is cheap, simple and well standardized, with it isolates can only be classified as sensitive or resistant based on the size of the inhibition zones [8]. The method is based on the principle of diffusion of plant extract into a solid nutrient medium,whereby it has a more or less inhibitory effect on the bacteria previously seeded on that substrate. Cellulose discs are applied, on which the sample extracted from the plants is applied. Cultures of microorganisms are seeded in Petri dishes. 1 ml of the bacterial culture suspension is applied evenly on the substrate with a stick according to Drigalski. Then cellulose discs soaked with extracts of the plants Parsley - *Petroselinum crispum* L., Garlic - *Allium sativum* L. and Turmeric - *Curcuma longa* L. are placed. The samples prepared in this way were incubated (Incubator Selecta: 2000209, Spain). The extract diffuses from the application site into the substrate in all directions. Preventing the growth of the tested microorganism occurs if it is sensitive to the effect of the tested sample. The width of the inhibition zone (inhibition diameter) is proportional to the degree of sensitivity of the microorganism to the applied agent, that is, to the antimicrobial effect of the examined extract (table 1). After incubation at 37°C, the first inhibition reading was taken after 24 hours, and the final results after 48 hours. If there is no inhibition zone, it means that the microorganism is not resistant to the effect of the tested substance. The experiment was performed in replicate with two types of control: positive and negative.

The negative control implies that water is applied to the disc instead of the sample in a certain dilution. Positive control implies the use of antibiotics. An antibiotic (Penicillin) was used as a control to compare the activity of the extracts.

Table 1. Resistance of bacteria towards microorganisms

Inhibition zone	Sensitivity marks	Interpretation
do 10 mm	R*	Resistant bacteria
10 - 17 mm	I, 2**	Moderately resistant bacteria
> 17 mm	S, 3***	Resistant bacteria

*R – resistant, **I – intermediate, ***S - sensitive

Data collected through experimental research were processed in a statistical program (PAST 4.0) and SAS 9.4 (SAS, 2012).

RESULTS AND DISCUSSION

The excessive use of antibiotics has led to the selection of new strains of bacteria resistant to antibiotics, a situation that is often encountered in practice, especially in the case of *Escherichia coli*. Mos et al. [9] investigated the sensitivity of different strains of Escherichia coli to different groups of antibiotics, including penicillin. They used two methods for the research: the disc diffusion method and the agar dilution method, and 113 strains of *Escherichia coli* were isolated from the external wounds of patients in the period from 2006 to 2008 and were used as the test material. The results confirm the low sensitivity of the bacteria to pencillins, and resistance of over 50%, which is in line with our research. The data that testifies to the increase of various phenotypes of bacteria resistant to multiple drugs in enterobacteriaceae, including *Escherichia coli*, are worrying, such as research [10]; [11]; [12]; [13]; [14]; [15]; especially third-generation cephalosporins, as well as colistin (used as an antibiotic of last resort to treat carbapenem-resistant enterobacteriaceae).

Statistical processing of the collected data from our research and the use of extracts of three plants (parsley, turmeric and garlic) determined their antibacterial effect on the tested bacteria. which characterizes it as an extract to which the tested bacterium is very sensitive because the identified inhibition zones are higher compared to the aforementioned studies. (table 2), but also a significant reaction of turmeric extract with a medium inhibition zone of 20,26 and garlic extract 19,63 mm.

Table 2. Results of inhibition zones - *Escherichia coli* ATCC 25922

Escherichia coli	Min.	Max.	Mean	Variance	Stan.dev.	Coeff.var.	Std.error
Petroselinum crispum	17,20	23,70	21,67	9,161	3,026	13,96	1,513
Curcuma longa	15,72	23,48	20,26	10,981	3,313	16,35	1,656
Alliuma sativum	17,82	23,17	19,63	5,947	2,438	12,42	1,219
ANOVA	Sum of sqrs		df	Mean squere		F	p (≤0,01)
Between groups	8,68805		4	4,34402		0,4995	0,062*
Within the group	78,9586		30	8,69673			0,9015

Kruskal - Walis test

H(ch^2): 1,192

H_c (tie corrected): 0,892

p (≤0,01): 0,05509

Statisticaly significant diference beetwen samples is present.

Fereshteh et al. [16] studying the antimicrobial activity of parsley (*Petroselinum crispum*) against pathogenic bacteria in food, conducted experimental research with the aim of determining the antimicrobial activity of the essential oil from the leaves and seeds of parsley.

Antimicrobial activity was tested using the paper disk diffusion method and the by micro dilution technique against five pathogenic bacteria (*Escherichia coli*, *Salmonella ssp.*, *Staphylococcus aureus*, *Yersinia* and *Vibrio cholera*).

The results show the high efficiency of essential oils in the control of pathogenic bacteria and the need for their use in the development of new systems for the prevention of bacterial growth in accordance with their role in extending the expiration date and increasing the safety of processed food. Parsley seed essential oil showed an inhibition zone diameter of 11 mm against Staphylococcus aureus, 10 mm against Escherichia coli and Yersinia, 10,5 mm against *Vibrio cholera*, while the diameter of the inhibition zone was low against *Salmonella* (9 mm). The diameter of the zone of inhibition of the essential oil from the leaves varied from 12 to 14,5 mm and is equal to the resistance of microorganisms to antibiotics currently in use. A large number of side effects during application, as well as the high price of synthetic compounds, resulted in the tendency of many researchers to search for efficient natural products. Kasta [17] investigating the necessary concentration and antimicrobial activity of the ethanolic extract of the rhizome of turmeric (*Curcuma Longa* L.) for the growth of *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* used Disk diffusion method to measure the zone of inhibition with different concentrations of turmeric rhizome extract (500 mg/mL, 400 mg/mL, 300 mg/mL, 200 mg/mL, 100 mg/mL, 50 mg/mL and 25 mg/mL). The results of phytochemical screening showed that the ethanolic extract of turmeric rhizomes contains alkaloids, flavonoids, saponins, tannins and triterpenoids/steroids, while the antimicrobial inhibition of the ethanolic extract of turmeric rhizomes against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* at a concentration of 500 mg/mL was 15,88 mm, 15,63 mm and 15,22 mm. Investigating the comparative assessment of the antimicrobial effects of garlic (*Allium sativum*) and antibiotics on diarrhoeagenic organisms, Egbobor [18] performed antimicrobial susceptibility tests on *Escherichia coli*, *Shigella sp*., *Salmonella sp*. and *Proteus mirabilis* using standard procedures.

Significant statistical differences at the level of significance (p<0,01) were observed in the action of antimicrobial agents (garlic, ciprofloxacin and ampicillin), and in the sensitivity of microbial species (p<0,01) to antimicrobial agents, which is in accordance with our conducted research where the average zone of inhibition was determined to be 19,65 mm. From the three tested cultures, the lowest inhibition zone on *Escherichia coli* was recorded with the use of garlic extract, but still sufficient to characterize that extract as favorable and significant in the fight against the mentioned bacteria with an inhibition zone greater than 17 mm and characterized as ***S - sensitive .

Salmonella can be obtained from any food that has not been handled properly and hygienically or that has not been properly processed. Symptoms can appear six hours to six days after infection and can last four to seven days, and include: abdominal cramps and pain, sudden fever, nausea, vomiting, headache, dehydration, and blood in the stool.

Microorganism resistance is still a challenge for health and food production. Based on the demand for natural products for the control of microorganisms, and the re-evaluation of potential medicinal plants for the control of diseases, the aim of this research was, among other things, to determine the antibacterial activity of parsley extract against the mentioned pathogen, but also the activity of *Curcuma longa* and *Allium sativum* both on *Salmonella enteriditis*, and the results are presented in table 2.

Table 3. Results of inhibition zone of *Salmonella enteritidis* ATCC 13076

Escherichia coli	Min.	Max.	Mean	Variance	Stan.dev.	Coeff.var	Std.error
Petroselinum crispum	15,79	16,96	16,375	0,294	0,542	3,344	0,2713
Curcuma longa	17,41	22,47	19,944	5,429	2,330	12,228	1,1665
Alliuma sativum	15,53	21,54	18,535	6,841	2,615	14,734	1,3078
ANOVA	Sum of sqrs		df	Mean squere		F	p (≤0,01)
Between groups	16,0806		4	8,04031		1,92	0,020**
Within the group	37,6962		30	4,18847			0,105

Kruskal - Walis test
$H(ch^2)$: 3,092
H_c (tie corrected): 1,179
p (≤0,01): 0,03209
Statisticaly significant diference beetwen samples is present.

Curcumin is the most important fraction of turmeric that is responsible for its biological activity. In the research of [19] the isolation and biological evaluation of turmeric and curcumin in relation to the standard and mycobacterial strains such as: *E. coli, S. aureus, E. feacalis, P. aeuroginosa, M. smegmatis, M. simiae, M. kansasii, M. terrae, M. szulgai* and fungi *Candida albicans*. All isolated turmeric extracts and curcumin showed very weak activity against the studied mycobacteria *M. smegmatis, M. simiae, M.*

kansasii, *M. terrae and M. szulgai*, and moderate antibacterial activity of the extract and pure curcumin against the gram-negative bacteria *E. coli* and *P. aeuroginosa*. The common onion, *Allium cepa*, in addition to its nutritional effects, has antibacterial and antifungal activity, while compounds obtained from onions have shown anti-inflammatory and antihistaminic effects in vitro and by applying them to animals [20]; [21]. The first studies prove antibacterial activity (including *H. pylori*), that is, antiparasitic and antifungal activity, which is in accordance with numerous studies, such as [23]; [24]; [25]; [26]. Conducted research by a group of Asian scientists on the topic: Antibacterial activity of parsley (*Petroselinum crispum*) and ethanolic extract of *Prunus mahaleb* seeds [27] where the antimicrobial activity against eleven bacteria (*Bacillus anthracis, Bacillus subtilis, Bacillus pumilus, Staphylococcus aurelia*, *Salmonella enterides*, *E. typhi*, *Proteus mirabilis, Bordetella bronchiseptica*, *Pseudomonas aeruginosa*) proved the antimicrobial activity of the tested plants. Based on the results, they proved that both plants can be considered disinfectants or antiseptics, which confirms their use in folk medicine, because the extracts are in different concentrations (0,1, 0,2, 0,3 and 0,4 g mL-1) showed an inhibitory effect on Gram-positive and Gram-negative bacteria. The ethanol extract of prunus mahaleba showed antibacterial activity *against P. mirabilis, B. anthracis and S. aureus, B. licheniformis* while a satisfactory inhibitory effect was found with parsley extract and action on *Br. melitensis*, *E. coli*, *B. licheniformis* and *Salmonella enterides* in low concentrations (0,1 and 0,2 g mL^{-1}), which is in accordance with our research.

A gram-positive, polymorphic, rod-shaped bacterium, *Listeria monocytogenes*, was first described in 1926 by Murray, Webb and Swann at the University of Cambridge. It was identified as the causative agent of the disease in laboratory animals (rabbits and guinea pigs) in 1924. The disease was manifested by a high number of mononuclear leukocytes (monocytosis). Today, listeriosis is a rare but serious disease caused by the bacterium *Listeria monocytogenes*, which can survive and grow on certain high-risk foods. Its characteristic features, such as reproduction within a wide range of pH and temperature, and its strong resistance to high concentrations of sodium chloride allow listeria to survive various processes used in food processing. If we add to this the ability to multiply at storage temperatures (+4°C), it is not surprising that this bacterium represents a significant problem for the food industry, and a potential danger to human health. Our conducted research did not record the influence of the tested extracts on *Listeria monocytogenes*, the established inhibition zones are very low values (3,33 mm).

The research is in line with the research of [28] who examined the antimicrobial activity of other plants: Thymus capitatus (L.) *Origanum elongatum* (Bonet) and *Mentha suaveolens* Ehrh., and also achieved very low zones of inhibition in the value of 3,01 mm, which is in accordance with our results.

Similar research was carried out by [29] in order to evaluate the effectiveness of five plant essential oils: thyme, mint, bay, sage and orange oils as natural food preservatives. The effect of plant essential oils on *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Candida albicans* in concentrations of 5 to 20 micro L/disc (diameter 6 mm) and 0,5-3% (v/v) was studied in agar diffusion test medium . The essential oils of these extracts showed an extremely antibacterial and bacteriostatic effect, with thyme showing the highest inhibition, and orange the lowest, but still not high enough to characterize the bacteria as sensitive.

CONCLUSION

Significant statistical differences at the level of significance ($p<0.01$) with a medium zone of inhibition of 21,67 mm prove the antimicrobial activity of parsley, and the sensitivity of *Escherichia coli* to the tested plant. Turmeric extract with an average inhibition zone of 22,26 and garlic extract 19,63 mm showed a significant reaction of the examined extracts to *Escherichia coli*, and they were characterized as ***S - sensitive.

With an inhibition zone of 19,052 mm on average, Curcuma longa showed the strongest effect on the tested bacterium *Salmonella enteritidis*, which tells us that it is a very sensitive bacterium to the extract of the mentioned plant. Microorganism resistance is still a challenge for health and food production, and based on the demand for natural products to combat microorganisms, we conclude that the garlic extract has a statistically significant antimicrobial activity on the tested bacteria with an average zone of inhibition of 17,75 mm and parsley extract 16,22 mm. The research determined very low inhibition zones (> 8mm) in all three examined extracts: *Allium sativum* L., *Curcuma longa* L. *Petroselinum crispum* L. on *Listeria monocytogenes*.

The obtained results of the experimental research are the basis for further research on the topic of the influence of solvents on the antimicrobial activity of the extracts of the tested plants, because the presented results of numerous scientists prove that the daily consumption of the aforementioned herbs improves the organoleptic properties of foods, but also reduces the population of tested pathogens in food, and this also improves people's health.

REFERENCES

[1] Anand, S. P.; Sati, N. „Artificial Preservatives and their harmful effects: Looking toward nature for safer alternatives‟. Int. J. Pharm. Sci. Res. 4(7):2496-2501, 2013.

[2] Silva, N.; Alves, S.; Gonçalves, A.; Amaral, J.S.; Poeta, P. „Antimicrobial activity of essential oils from Mediterranean aromatic plants against several foodborne and spoilage bacteria‟. Food Science and Technology International, 19(6): 503-510, 2013.

[3] Josipović, R.; Markov, K.; Frece, J.; Stanzer, D.; Cvitković, A.; Mrvčić, J. „Upotreba začina u proizvodnji tradicionalnih sireva‟. Mljekarstvo, 66 (1): 12-25, 2016.

[4] Melvin, Joe. M.; Jayachitra, J.; Vijayapriya, M. „Antimicrobial activity of some common spices against certain human pathogens‟. Journal of Medicinal Plants Research Vol. 3(11), 1134-1136, 2009.

[5] Tufail, M. „Spicies in Indian Economy‟. Academic Foundation California University 151, 1990.

[6] WHO. „Global action plan on antimicrobial Resistance‟. http://www. wpro.who.int/entity/drug_resistance/resources/global_action_plan_eng.pdf. Accessed 14 Oct 2021. 2019.

[7] Jorgensen, M. J.; Ferraro, J. H. „Antimicrobial Susceptibility Testing: A Review of General Principles and Contemporary Practices‟. Clinical Infectious Diseases, 7750, 1749-1755, 2009.

[8] *EFSA* - European Food Safety Authority. „Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance‟. EFSA Journal, 10(6), 1-10, 2012.

[9] Moş, Ioana.; Otilia, Micle.; Mihaela, Zdrâncă.; Mariana, Mureşan.; Laura, Vicaş. „Antibiotic sensitivity of the *Escherichia coli* strains isolated from infected skin wounds‟. Farmacia, 2010, Vol.58, 637-645, 2010.

[10] Borg, M.A.; Scicluna, E.; De Kraker, M.; Van De Sande-Bruinsma, N.; Tiemersma, E.; Gür, D.; Ben, RS.; Rasslan, O.; Elnassar, Z.; Benbachir, M. „Antibiotic resistance in the southeastern Mediterranean–preliminary results from the ARMed project‟. Euro Surveill. 2;11:164-167, 2006.

[11] Burow, E.; Simoneit, C.; Tenhagen, BA.; Käsbohrer, A. „Oral antimicrobials increase antimicrobial resistance in porcine *E. coli*–a systematic review‟. Prev Vet Med.;113:364-374, 2014.

[12] Cannatelli, A.; Giani, T.; Antonelli, A.; Principe, L.; Luzzaro, F.; Rossolini, GM. „First detection of the mcr-1 colistin resistance gene in *Escherichia coli* in Italy‟. Antimicrob Agents Chemother.; 60:3257-3268, 2016.

[13] Hasman, H.; Hammerum, A.M.; Hansen, F.; Hendriksen, R.S.; Olesen, B.; Agersø, Y.; Zankari, E.; Leekitcharoenphon, P.; Stegger, M.; Kaas, RS. „Detection of mcr-1 encoding plasmid-mediated colistin-resistant *Escherichia coli* isolates from human bloodstream infection and imported chicken meat‟. Denmark. Euro Surveill. 20:1-5, 2015.

[14] 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. Antimicrob Agents Chemother.;60:4420-4431.

[15] Poirel, L.; Kieffer, N.; Liassine, N.; Thanh, D.; Nordmann, P. „Plasmid-mediated carbapenem and colistin resistance in a clinical isolate of *Escherichia coli* ‟. Lancet Infect Dis.;16:00006–2, 2016.

[16] Fereshteh, K.; Mohammad, R.; Nabi, S.; Mehran, S.; Issa, M.; Ebadallahshiri, M.; Hassan, J. Running Head: „Antimicrobial activity of Parsley Antimicrobial Activity of Essential oil of Parsley (*Petroselinum crispum*)„. Against Food Pathogenic Bacteria World Applied Sciences Journal 31 (6): 1147-1150, 2014.

[17] Kasta, Gurning. „Antimicrobial Activity of Ethanol Extract of Rhizome Turmeric (*Curcuma Longa* L.) For Growth of *Escherichia coli, Staphylococcus aureus* and *Candida albicans*‟. Asian Journal of Pharmaceutical Research and Development. 8(3):05-08, 2020.

[18] Egbobor, Eja.; Matthew, Bassey.; Asikong, E.; Clement, Abriba.; Giddings, E.; Arikpo, Edet E.; Anwan, Kingsley.; H, Enyi-Idoh. „A Comparative assessment of the antimicrobial effects of garlic *(Allium sativum*) and antibiotics on diarrheagenic organisms southeast‟. Asian trop Med Public Health vol 38 No. (2), 343-348, 2007.

[19] Çıkrıkçı, Simay.; Erkan, Mozioğlu.; Hasibe, Yılmaz. „Biological Activity of Curcuminoids Isolated from *Curcuma longa*‟. Rec. Nat. Prod. 2:1,19-24, 2008.

[20] WHO. „Monographs on selected medicinal plants‟. Geneva, Switzerland 1: 5, 1999.

[21] Griffiths, G.; Trueman, L.;Crowther, T.; Thomas, B.; Smith, B. „Onions-a global benefit to health‟. Phytother. Res., 16: 603-615, 2002.

[22] Celikel, N.; Kavas, G. „Antimicrobial properties of some essential oils against some pathogenic microorganisms‟. Czech J. Food Sci., 26: 174-181, 2008.

[23] USDA. „The PLANTS database‟. National Plant Data Center, Baton Rouge, LA 70874-4490 USA. (http://plants.usda.gov/July 2010), 2007.

[24] Rose, P.; Whiteman, M.; Moore, PK.; Zhu, YZ. „Bioactive S-alk (en)yl cysteine sulfoxide metabolites in the genus *Allium*: The chemistry of potential therapeutic agents‟. Natural Prod. Rep., 22: 351-368, 2005.

[25] Elnima, E.I.; Ahmed, S.A.; Mekkawi, A.G.; Mossa, J.S. „The antimicrobial activity of garlic and onion extracts‟. Pharma., 38: 747-748, 1983.

[26] Zohri, A.N.; Gawad, KA.; Saber, S. „Antibacterial, antidermatophytic and antioxigenic activities of onion (*Allium cepa*) oil‟. Microbiol. Res., 150: 167-172, 1995.

[27] Sevvedneiad, S.M.; Maleki, S.,; Damb, N.M.; Motamed, H. „Antibacterial Activity of Prunus mahaleb and Persley (Petroselinum crispum) Against Some Pathogen‟. Asian Journal of Biological Sciences Volume 1, 51-55, 2008.

[28] Naim, El, Moussaoui.; Gloria, Sanchez.; El Ouardy, Khay.; Mohammed, Idaomar.; Ahmed, Ibn Mansour.; Jamal, Abrini.; Rosa, Aznar. „Antibacterial and Antiviral Activities of Essential Oils of Northern Moroccan Plants‟. British Biotechnology Journal (3): 318-331, 2013.

[29] Celikel, N.; Kavas, G. „Antimicrobial properties of some essential oils against some pathogenicmicroorganisms Czech‟ J. Food Sci., 26:174-181, 2008.