An Investigation into the Effect of Aqueous Extract of Garlic (*Allium-sativum*) on Growth Inhibition of *Escherichia coli* Isolated from calves with Diarrhea in Kazerun

Sayahi, A.[1]; Nowroozi-Asl, A.[2*]; Fattahi, H.[3]

[1]Young and Elite Research Club, Kazerun Branch, Islamic Azad University, Kazerun, Iran; [2]Department of Clinical Sciences, Faculty of Veterinary Medicine, Kazerun Branch, Islamic Azad University, Kazerun, Iran; 3Department of Pathobiology, Faculty of Veterinary Medicine, Kazerun Branch, Islamic Azad University, Kazerun, Iran.

[*]**Corresponding Author:** A. Nowroozi-Asl, Department of Clinical Sciences, Faculty of Veterinary Medicine, Kazerun Branch, Islamic Azad University, Kazerun, Iran, *E-mail:nowroozia@yahoo.com*

(Received: September 15, 2017; Accepted: November 30, 2017)

Abstract

Diarrhea in calves is one of the most prevalent diseases the veterinarians encounter in practice. Diarrhea is responsible for major economic damages in livestock husbandries. *Escherichia coli* (*E.coli*) has been regarded as the main factor that causes white diarrhea in calves' first three weeks of life. Antimicrobial treatment of *E. coli* is difficult since these bacteria naturally show resistance against most antibiotics. The aim of the present study is to investigate the antibacterial activity of aqueous extract of garlic on *Escherichia coli* isolated from calves with diarrhea. This descriptive cross-sectional study was carried out to investigate the effect of aqueous extract of garlic on 100 samples of calves with diarrhea collected from traditional and industrial livestock husbandries crops in Kazerun during February and March 2016 and April 2017. The isolates susceptibility to different antibiotics was tested by the disc diffusion method and the antibacterial activity of garlic extract was measured by MIC and MBC methods. In this study, the highest resistance was seen against ampicillin and tetracycline (0± 0.4 mm, 0± 0.5 mm) and the lowest resistance was against ciprofloxacin (40 ± 2/54 mm). The halo diameter formed by aquatic extract of garlic was similar to streptomycin (15 ± 1/00 mm). In general, the results showed that garlic has the potentiality to be used as an antimicrobial drug, especially in the treatment of *E. coli*-induced diarrhea in calves.

Key words: aquatic garlic extract, calf diarrhea, *Escherichia coli*, Kazerun

Introduction

Diarrhea in calves has been an important serious problem and is one of the major causes of economic losses in each husbandry due to their fatality, their inevitable treatment costs and a reduction in calves' growth (Andrews et al., 2004). Neonatal calf diarrhea is not associated with only an agent but it is a multi-factorial disease. The infections factors are mainly related to *E. coli*, salmonella, rotaviruses and coronaviruses, some protozoan organisms such as cryptosporidium and coccidia parasite (Shams et al., 2010). The *Escherichia coli* are the most

prevalent cause of diarrhea in calves in the first three weeks of their lives (Nagy and Fekete, 2005).

In recent years, commercially available anti-microbial drugs have been used to control infectious diseases. The excessive use of antibiotics has led to the development of multiple drug resistance in most bacterial pathogens (Erdogrul, 2002; Ouahouo et al., 2004). Concerning the increasing use of antibiotics and consequently the increased antibiotic resistance and different susceptibility to *E.coli* in different parts of the world, the aim of the present study was to determine the prevalence of *E.coli* in diarrhea of calves in a number of traditional and industrialized animal husbandries in Kazerun city through the culture method, utilizing aqueous extract of garlic instead of antibiotics.

Garlic (*Allium Sativum*), a plant belonging to the *Liliaceae* family, is native to Central Asia (Haciseferogullari et al., 2005). It has antibacterial, antiviral, antifungal, antioxidant and anti-inflammatory properties (Banerjee and Maulik, 2002; Peng, 2002). Moreover, garlic has been recognized as a strong antibacterial agent and its inhibitory effect on both gram-negative bacteria and gram-positive bacteria has been proven (Ankri and Mirelman, 1999; Sivam, 2001). According to a study by Shobana and his colleagues (Shobana et al., 2009), garlic extract inhibits intestinal pathogens such as *Escherichia*, *Proteus mirabilis*, *Salmonella typhi*, and *Shigella flexneri*. Sadeghian and Ghazvini (2002) also observed that all tested isolates of Shigella were susceptible to garlic extracts, showing no resistance to these extracts. In a study on 289 *E. coli* samples isolated from calves' faeces, Blanco and some other researchers (1988) reported that 20.5% of the samples were Verotoxigenic *Escherichia coli* (VTEC). Further, the effects of these bacteria on rats were examined.

Using culture medium, Fukushima and his colleagues (2004) studied 605 bovine feces samples in order to detect shiga toxin-producing *Escherichia coli*. The reported that 31 strains (1.5%) were infected with Verotoxigenic *Escherichia coli*.

In his research, Bulgin and others (1982) observed that 20% of the female dairy calves and 12% of males were infected with diarrhea recognized as multifactorial. They also regarded multifactorial issue of diarrhea in calves as normal. The prevalence of enterotoxicogenic *E.coli* in calves with diarrhea varies in different herds according to their age in different areas. (Bulgin et al., 1982).

Materials and Methods

In this research, 100 samples of feces swabs from calves (under one month of age) with clinical diarrhea were collected from some husbandries in Kazerun. The swabs were inserted into the tubes containing Selenite F Broth and transferred to a microbiology laboratory at Islamic Azad University, Kazerun branch.

After completion of sampling, the enrichment of samples was done on Selenite *F* Broth and isolation on MacConkey Agar, MAC (Lab. M) containing 0.05 mg/l Cefixime (Oxoid company), and 2.5 mg/l potassium tellurite (Oxoid) at 37 ° C for 24 hours. To evaluate the lactose fermentation and to determine the identity of isolated bacteria, Eosin Methylene Blue agar, EMB (Merck, Germany) was used (Islam et al., 2007; Park et al., 2002; Rivas et al., 2003).

In this study, aqueous extract of garlic was prepared according to Bucker and Douglas (2005). In this method, fresh garlic were washed, peeled, cleaned, and sliced, and then dried for 7 days on the sunny day (shade). Garlic slices were then homogeneously powdered with an electric mill. 10 grams of this powder was transferred to a container containing 100 sterile distilled water. The dish was incubated for 48 hours at room temperature under shaking conditions (rotary shaker) at 120 rpm. The extract was centrifuged for 20 minutes at 6000 rpm at 25 °C., and then filtered with a Whatman No. 1 filter paper. The final concentration of garlic extract was measured at 100 mg / ml and it was stored at 4 ° C.

The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were determined using dilution method in the tube. To determine MIC, a series of 11 test tubes were used for extraction. 9 test tubes were used to test different dilutions of each extract, one tube as positive control (containing diluted extract plus culture medium) and a tube as negative control (containing microbial suspension plus culture medium) (Aksoy et al., 2006; Ayepola and Adeniyi, 2008; ISIRI 6806/1, 2005; Sindambiwe et al., 1999).

[DOR: 20.1001.1.2717378.2017.1.3.2.2]

Results and Data Analysis

Of the total samples in this study, 36 samples (36%) were related to male calves and 64 (64%) were related to female calves (Chart 1).

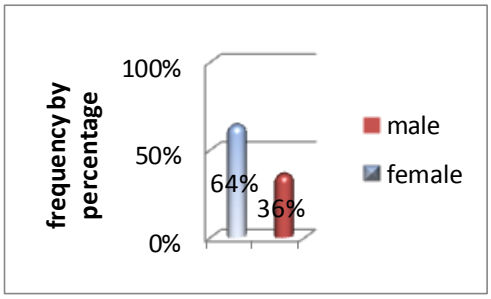

Chart 1. The sample frequency

The absolute and relative frequency distribution of the population under study based on the age is presented in Table 1.

Table 1. Absolute and relative frequency distribution of the population based on age

Age (Day)	0-5	6-10	11-15	16-20
Frequency	17 (17%)	33 (33%)	35 (35%)	15 (15%)

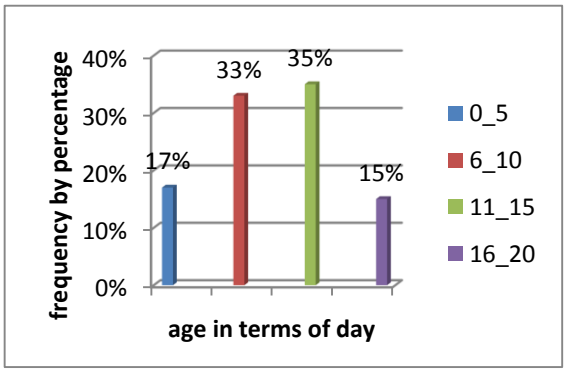

Chart 2.The frequency of sample population in terms of age

Absolute and relative frequency distribution of the population under study based on gender has been presented in Table 2.

Table 2. Absolute and relative frequency distribution of the population based on gender

Age(day)	male	Female
0-5	5(13.89%)	12(18.75%)
6-10	10(27.77%)	**23(35.93%)**
11-15	14(38.89%)	**21(32.81%)**
16-20	7(19.44%)	**8(12.5%)**
Total	36	64

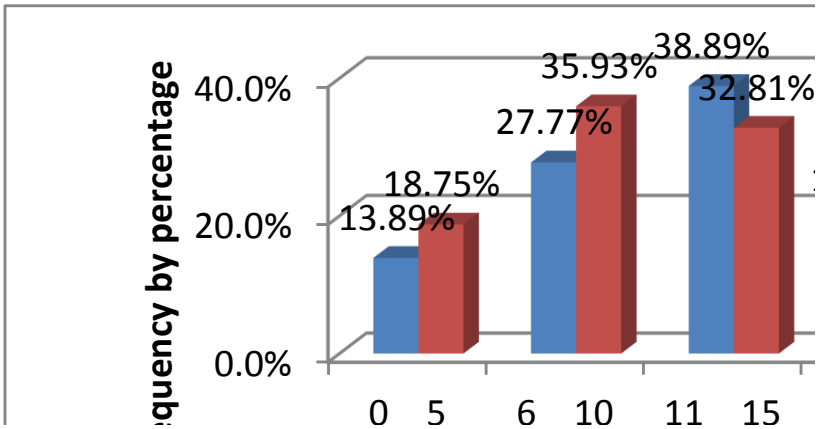

Chart 3. Comparing *E.coli* contamination rate between male and female

The calves diarrhea samples were cultured on mechanical culture medium, the bacterial growth was observed on 70 plates while pathologic bacteria did not grow on 30 plates. Then, these bacteria were cultured on EMB(Eosin Methylene Blue agar) in which 43 colonies in a bright metallic green color were grown after 24 hours of incubation at 37℃, indicating the presence of *E.coli* bacteria.

[DOR: 20.1001.1.2717378.2017.1.3.2.2]

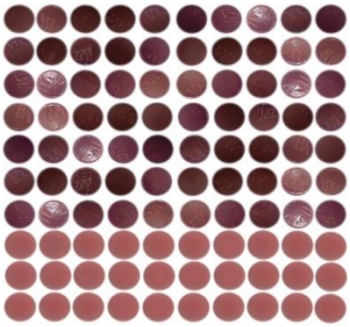

Figure 1. Culture of calves diarrhea samples on a MacConkey culture medium

Figure 2. Culture of calves diarrhea samples on EMB

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) is the minimum concentration that can prevent the bacterial growth up to 90%. The results obtained from the minimum inhibitory concentration of aqueous extract of garlic are presented in Table 4. The lowest antibacterial concentration of aqueous extract of garlic on *Escherichia coli* was found as 6.25 mg/ml (Table 4).

Table 4. The result of MIC test in different tubes

Tubes No.	Extract Concentration	Garlic Extract
1	50	+
2	25	+
3	12.5	+
4	6.25	+

5	**3.125**	**-**
6	**1.562**	**-**
7	**0.781**	**-**
8	**0.390**	**-**
9	**0.195**	**-**
10	**0.097**	**-**

Minimum bactericidal concentration (**MBC**)

The minimum bactericidal concentration *(*MBC*)* is the minimum concentration of extract that prevents bacterial growth up to 99.9%. The evaluation of MBC test was carried out for concentrations 6.25, 12.5, and 25 mg/ml and the results showed a progressive growth of the bacteria.

Tube number 4 (concentration 6.25 mg/ml) | Tube number 3 (concentration 12.5mg/ml) | Tube number 2 (concentration 25 mg/ml)

Figure 3. The results of MBC on aqueous extract of garlic

Agar diffusion with disk

[DOR: 20.1001.1.2717378.2017.1.3.2.2]

The results for the diameter of the growth inhibition zone are shown in Table 5. The aqueous extract of garlic on the growth of *E. coli* showed inhibitory effect (15 mm). All antibiotics used on *Escherichia coli* bacteria were effective except for ampicillin and tetracycline which showed resistance. The highest effect belonged to ciprofloxacin and gentamicin (40 and 30 mm) and the least effect was related to streptomycin (15 mm).

Table 5.The results of antibiotic gram

Antibiotic	**Holo Diameter in Galic Plates**
Cefixime	20±1/52mm
Chloramphenicol	22±1/46mm
Nitrofurantoin	25±1/31mm
streptomycin	15±1/15mm
Tetracycline	0±./5mm
Nalidixic acid	23±1/63mm
Ampicillin	0±./4mm
Ciprofloxacin	40±2/54mm
Gentamicin	30±2/08mm
Garlic extract	15±1/00mm

[DOR: 20.1001.1.2717378.2017.1.3.2.2]

Figure 4. Results of diffusion disk for garlic extract

[DOR: 20.1001.1.2717378.2017.1.3.2.2]

Discussion

In the present study, using 100 samples of calf diarrhea isolated from animal husbandries around Kazerun through culture method, 43 samples (43% of the cases) were *E. coli*.

Similar studies in some other areas have reported comparable results with the present research findings. In 1988 in northwestern Spain, Blanco and his colleagues studied a total of 289 colonies isolated from diarrheic calves and reported that 20.5% of samples were *E. coli* (Blanco et al., 1988). In Japan, Fukushima and some other researchers (2004) investigated 605 cattle (caws and calves) feces, using culture and PCR method, to search for Shiga toxin-producing *Escherichia coli* (STEC). As they reported, 31 of samples were infected with *Escherichia coli* (Fukushima and Seki, 2004).

Different cases of *E.coli*-induced diarrhea have been reported in Iran. In a study by Shams and his colleagues conducted through using mPCR in Fars province, 5.3% were reported as *E. coli* diarrhea (Shams et al., 2010). On the other hand, using agglutination method in Babol and Ghaemshahr was reported this rate as 1.07% (Lotfollahzadeh et al., 2005). It seems that some of

the disparities are related to the differences in the prevalence rate of *Escherichia coli* diarrhea in these areas. Moreover, the sensitivity and specificity of the methods used are also different.

The antibiogram test also indicated that the isolated *Escherichia coli* were susceptible to most antibiotic groups used in the treatment of diarrhea. This drug susceptibility was found to be less frequently in traditional animal husbandries compared to the industrial ones. It might be due to the uncontrolled and constant use of a certain drug or the lack of therapeutic doses of antibiotics. Bulgin and his colleagues (1982) also observed that Salmonella and *Escherichia coli* were resistant to certain drugs in laboratory conditions, especially those used to treat calves diarrhea. In this study, *E. coli* isolated from dairy farms from around Kazerun was not susceptible to tetracycline and ampicillin.

Breuer's (2001) studies in America, Radu (2001) in Malaysia, Kim (2005), Kargar (2005) in Jahrom, Nahaie (2007) in Tabriz also reported isolated strains resistance to ampicillin which were in line with findings of this present study. On the other hand, kanamycin resistance in 1996 in Japan (Kim et al., 2005), *erythromycin resistance* in 2005 in Korea (Kim et al., 2005) and penicillin resistance in 2005 in Jahrom have been reported, the reason for this disparities could be due to the climatic conditions.

Garlic is one of the most important aromatic herbs with a wide range of antimicrobial activity against a variety of microorganisms, including pathogens and corrosion agents or food poisoning (Aneela et al., 2001; Shobama et al., 2009). The active ingredient in garlic is a sulfur compound called allicin. Allicin is a chemical substance found in the crushed or chewed garlic. Allicin is very potent used as an antibiotic that helps control microbial growth and replication, with an antiviral, fungal, bacterial and antioxidant properties.

It seems that allicin and its thiol-disulfide exchange reactions with free thiol groups of various enzymes are a mechanism that leads to all these activities. Therefore, the antibacterial effect of cooked garlic and commercially available garlic pills is less than the effect of inhibitory effect of the raw garlic (Ankri and and Mirelman, 1999; Miron et al., 2002).

Alzowahi and his colleagues (2013) examined the effect of garlic extract on some enterobacteriaceae species. They observed that MIC of this extract on Salmonella, Enterobacter, and *Escherichia coli* species were 6.25%, 12.5% and 1.56%, respectively. Daka and Awole (2009) also evaluated the antibacterial performance of aqueous extract of garlic on *E. coli*,

[DOR: 20.1001.1.2717378.2017.1.3.2.2]

Shigella strains and Salmonella strains, and found that MIC of this extract for all tested organisms was higher than 30 mg / ml but its MBC values for Shigella and Salmonella were 30 mg / ml and for *Escherichia coli* 5.37 mg / ml. In addition, Gull and other researchers (2012) studied the antimicrobial activity of aqueous, ethanolic and methanolic extracts of garlic and found that both tested gram-positive and gram-negative bacteria were susceptible to the extracts under study, but the susceptibility of the gram-positive bacteria to these extracts were higher. They also observed that the aqueous extract of garlic demonstrated significant antibacterial activity to all tested bacteria except *Escherichia coli* and Shigella. Shobana and others (2009) also stated that low concentrations of aqueous garlic extract inhibit the growth of intestinal pathogenic bacteria such as *Escherichia coli*, Salmonella typhi and Shigella flexneri. Durairaj and his colleagues (2009) studied the antimicrobial effects of aqueous garlic extract on 17 bacterial isolates with multiple drug resistance and observed that MIC range of this extract on gram-negative and gram-positive bacteria was 7-21 mg / ml and 6-11mg / ml.

Siwam (2001) also examined the antibacterial effects of garlic extract on Helicobacter pylori, which is associated with gastric ulcers and gastric cancer, and stated that this extract has synergistic effects on antimicrobial activity of omeprazole and on the tested bacterium.

The results reported by some other researchers (Alzowahi et al., 2013) are highly in line with the findings of this study. In contrast, the levels of MICs reported by Daka and Awole (2009) were higher and MIC values observed by Shobana and his colleagues (2009) were lower than the MICs obtained in the present study.

Conclusion

The findings in this study showed that garlic extract concentrations that were effective on *E.coli* varied from the concentrations obtained on other gram-negative bacteria, which were in line with the results of the previous studies (Alzowahi et al., 2013; 1Gull et al., 2012).

Overall, the results of this study revealed that garlic has the potentiality to be used as an antimicrobial drug, especially in the treatment of *E. coli*-induced diarrhea in calves. Though clinical experiments on patients are recommended after their garlic extract intake in order to confirm these data, it seems that further studies are needed to investigate the effects of this extract in intravenous conditions, to standardize it, and to conduct clinical evaluations. Finally,

this extract can be classified as a formulated herbal drug in veterinary pharmacies available to livestock breeders.

References

Aksoy, A., Duran, N., Koksal, F. (2006). In vitro and in vivo antimicrobial effects of mastic chewing gum against Streptococcus mutans and mutans streptococci. Archives of Oral Biology, 51:476-481.

Alzowahi, FAM., Abu-Taleb, A., As-Suhbani, A., Kadam, T.A. (2013). The inhibitory effects of garlic extract and its fractions against some Enterobacteriaceae spp. isolated from sprouted Mung bean. International Journal of Current Microbiology and Applied Sciences., 2: 104-115.

Andrews, A., H., Blowey R. W., Boyd, H., Eddy, R. G. (2004) Bovine medicine disease and husbandry of cattle. 2nd ed, Blackwell Publishing. pp: 186-230.

Aneela, F., Tauqeer, A., Shaistaj, K., Deeba, F., Zaidi, N. (2001). Assessment of antibacterial activity of in vitro and in vivo grown garlic (Allium sativum L.). Pakistan Journal of Botany., 43: 3029-3033.

Ankri, S. and Mirelman, D. (1999). Antimicrobial properties of allicin from garlic. Microbes and Infection, 1:125-129.

Arora, D.S., Kaur, J. (1999). Antimicrobial activity of spices International Journal of Antimicrobial Agents, 12: 257-62.

Ayepola, O.O., Adeniyi, B.A. (2008). The antibacterial activity of leaf extracts of Eucalyptus camaldulensis (Myrtaceae). Journal of Applied Sciences Research, 4:1410-1413.

Bakri, I.M., Douglas, C.W. (2005). Inhibitory effect of garlic extract on oral bacteria. Archives Oral Biology.,50: 645-51.

Banerjee, S.K., Maulik, S.K. (2002). Effect of garlic on cardiovascular disorders: a review. Nutrition Journal., 1:4.

Blanco, J., Gonzalez, E.A., Garcia, S., Blanco, M., Regueiro, B., Bernardez, I. (1988). Production of toxins by *Escherichia coli* strains isolated from calves with diarrehoea in Galicia (North-western Spain), Veterinary Microbiology, 18: 297-311.

Breuer, T., Benkel, D.H., Shapiro, R.L., Hall, W.N., Winnett, M.M., Linn, M.J. (2001). A multistate outbreak of *Escherichia coli* O157:H7 infections linked to Alfalfa sprouts grown from contaminated seeds. Emerging Infectious Diseases journal., 7: 977-82.

Bulgin, M. S., Anderson, B. C., Ward, A and Everman, J.F. (1982): Infections agents associated with neonatal calf disease in south western Idaho and eastern organ. Journal of the American Veterinary Medical Association., 180: 1222.

Daka, D. and Awole, M. (2009). Assessment of the antibacterial effect of crude preparation of garlic (Allium sativum) on diarrhea causing bacteria: an In vitro, study. Asian Journal of Medical Sciences., 1: 12-14.

Durairaj, S., Sangeetha, S., Lakshmanaperumalsamy,P. (2009). In vitro antibacterial activity and stability of garlic extract at different pH and temperature. Electronic Journal of Biology., 5: 5-10.

Erdogrul, O.T. (2002). Antibacterial activities of some plant extracts used in folk medicine. Pharmaceutical Biology., 40:269–273.

Fukushima, H. and Seki, R. (2004). High numbers of shiga toxin-producing *Escherichia coli* found in bovine feces collected at slaughter in Japan, FEMS Microbiology Letters, 238: 189-197.

Gull, I., Mariam, S., Halima, S., Shahbaz, M.A., Zahoor, Q.S., Amin, M.A. (2012). Inhibitory effect of Allium sativum and Zingiberofficinale extracts on clinically important drug resistant pathogenic bacteria. Annals Clinic Microbiol Antimicrobials, 11:8.

Haciseferogullari, H., Ozcan, M., Demir, F., Calisir, S. (2005). Some nutritional and technological properties of garlic (Allium sativum). Journal of Food Engineering., 68: 463-9.

ISIRI 6806/1: (2005). Microbiology of food and animal feeding stuffs – Enumeration of coagulase – Positive Staphylococci (Staphylococcus aureus and other species) – Test method Part 1: Technique using Baird – parker agar medium 1st ed., Karaj, pp: 404.

Islam, M.A., Heuvelink, A.E., de Boer, E., Sturm, P.D., Beumer, R.R., Zwietering, M.H. (2007). Shiga- toxin-producing *Escherichia coli* isolated from patients with diarrhoea in Bangladesh. Journal of Medical Microbiology, 56: 380-85.

Kargar, M., Heidary, S., Abbasian, F., Shekarforoosh, S. (2006). Survey of prevalence and antibiotic susceptibility anverotoxin production of *E.coli* verotoxigenic (*E.coli* O157:H7) in raw milk of Jahrom cows. Iranian Journal ofInfectious diseases and Tropical Medicine, 34: 7-11. (In Persian)

Kim, J., Kim, S., Kwon, N., Bae, W., Lim, J., Koo, H. (2005). Isolation and Identification of *Escherichia coli* O157:H7 UsingDifferent Detection Methods and Molecular Determination by Multiplex PCR and RAPD . Journal of Veterinary Sciences, 6: 7-9.

Lotfollahzadeh, S., ZiaeiDaroonkolai, N., ZahraeiSallehei, T., Poorbakhsh, S. A., MokhberDezfouli, M. R., Afshari, G.H. R. (2005) A study on the presence of *Escherichia coli*, coccidian and cryptosporidium in stool samples of under month age diarrheic calves in Ghaemshahr and Babol and antibiotic sensivity of isolates. Journal of the faculty of veterinary medicine university of Tehran, 59: 131-136. (In Persian)

Miron, T., Feigenbalt, G., Weiner, L., Mirelman, D., Wilchek, M., Rabinikov, A. (2002). A spectro- photometric assay for allicin, allin and alliinase (alliin-lyase) with a chromogenic thiol: reaction of 4-mercaptopyridine with thiosulfinates. Analytical Biochemistry, 307:76-83.

Nagy, N., Fekete, P. Z. (2005) Entrotoxigenic *Escherichia coli* in veterinary medicine. International Journal of Medical Microbiology, 295: 443–454.

Nahaei ,M.R., Akbari Dibavar, M., Sadeghi, J., Nikvash, J. (2007). Frequency of Enterohaemorrhagic *Escherichia coli* isolatedfrom patients with acute diarrhea in Tabriz hospitals. Indian Journal of Medical Microbiology., 3: 39-46. (In Persian)

Ouahouo, B.M., Azebaze, A.G., Meyer, M., Bodo, B., Fomum, Z.T., Nkengfack, A.E. (2002). Cytotoxic and antimicrobial coumarins from Mammea Africana. Annals of Tropical Medicine & Parasitology 2004; 98:733-739.

Park CH, Kim HJ, Hixon DL. Importance of testing stool specimens for shiga toxin. Journal of Clinical Microbiology, 40: 3542-43.

Peng, Q., BuzZard, A.R., Lau, B.H. (2002). Neuroprotective effect of garlic compounds inamyloid-beta peptide-induced apoptosis in vitro. Medical Science Monitor., 8:328–337.

Rivas, M., Caletti, M.G., Chines, I., Refi, S.M., Roldan, C.D., Chillemi, G. (2003). Home-prepared hamburger and sporadic hemolytic uremic syndrome. Argentina Emerging Infectious Diseases., 9: 1184-86.

Sadeghian, A., Ghazvini, K. (2002). Antimicrobial activity of garlic extract against Shigella. International Journal of Molecular Sciences (IJMS), 27: 142-144.

Shams, Z., Tahamyan, Y., Pourbakhsh, A., Hosseiny, M.H., Kargar, M., Hayati, M. (2010): Detection of entrotoxigenic K99 (F5) and F41 from fecal samples of calves by molecular and serological method, Comparative Clinical Pathology, Published online.

Shobana, S., Vidhya, V.G., Ramya, M. (2009). Antibacterial activity of garlic varieties (Ophioscordon and Sativum) on enteric pathogens. Current Research Journal of Biological Sciences., 1: 123-126.

Sindambiwe, J.B., Calomme, M., Cos, P., Totte, J., Pieters, L., Vlietinck, A. (1999). Screening of seven selected Rwandan medicinal plants for antimicrobial and antiviral activites.Journal Ethnopharmacol, 65: 71-77.

Sivam, G.P. (2001). Protection against Helicobacter pylori and other bacterial infections by garlic. Journal of Nutrition, 131: 1106S-1108S.

[DOR: 20.1001.1.2717378.2017.1.3.2.2]