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ARTICLE

A Comparative Investigation on Growth of Three Food Born Pathogenic Bacteria Inoculated with *Withania somnifera*: an Invitro Experimental Study

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ABSTRACT

Background: *Withania somnifera* (*WS*) is proposed as one of the alternatives instead of the antibiotic. This study is aimed to evaluate the inhibitory potency of enzymatic extract of the fruits of the *WS*. Methods: As an invitro experimental study, the growth rate of *Shigella dysenteriae, Salmonella typhimurium*, and *Escherichia coli* inoculated in different concentrations (25%, 12.5%, 6.25% and 3.125%) of the extract were assessed. A microtitre plate method was conducted. ANOVA was applied to identify statistical differences with *p*-value <0.05. Results: Different concentrations of extract, in comparison with control, declined the growth rate of all tested bacteria. All concentrations inhibited the growth of *S. typhimurium* (p<0.05). Compared to the microorganism control, effective concentration of the extract inhibiting the growth of *E. coli* was 12.5%, and 6.25%, while it was 12.5%, and 6.25% for *Sh. dysenteriae* (p<0.05). A dose-dependent response of *E. coli* was observed. The antibacterial activity of the extract tested was found mainly against *E. coli* and *Sh. dysenteriae*. The most resistant microorganism compared to *E. coli* and *Sh. dysenteriae* was *S. typhimurium* (*p*<0.05). 25% of the concentration of the extract showed the different inhibitory effect among three tested bacteria (*p*<0.05). Conclusions: The extract was labeled as an antibacterial agent against the representative of three foodborne bacteria, Invitro. The common effective concentrations of the extract (12.5, and 6.25%) is recommended for further research, as food additive, to remedy digestive ailments related to *E. coli*, *S. typhimurium* and *Sh. dysenteriae*.

1. Key Messages

The results of this study highlight the antibacterial effect of *Withania somnifera* (*WS*), a medicinal plant in Sistan and Baluchistan province, Iran, against *Shigella dysenteriae*, *Salmonella typhimurium*, and *Escherichia coli* (*E. coli*), as food born human/animal pathogens.

As a continuous work to our previous relevant study, the present study figures out the effect of different concentrations of crude enzymatic extract of *WS* berries against *E. coli*, *S. typhimurium* and *Sh. dysenteriae* growth, and also, register the local understanding of traditional medicines' use by residents in Sistan and Baluchistan.

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2. Introduction

Today, around 60% of anti-infective and antitumor medicaments, found in the market, composed of natural origin. [1] Medicinal herbs have fewer side effects, and are cheaper than chemical drugs, and also, easily available. [2] Statistics on the use of herbal medicines are significant in recent years. The World Health Organization revealed that about 4 billion people, now, use herbal medicine to treat illnesses. [3] Maybe, it is an unlikely explanation for many people that a variety of the chemical drugs in colorful packaging is the result of scrutiny on the effective elements of medicinal plants. This has led to form a widespread investigation by pharmaceutical companies on the healing properties of plants in different parts of the world. [3]

Designation of a national pharmacopeia, monographs of medicinal materials, standards and guidelines regarding medicinal herbs is suggested. [2] *WS* is considered as a medicinal plant in Sistan and Baluchestan province, Iran. [4,5] This plant is one of the vegetation of many cities of South of the province, including Saravan, Iranshahr, Sarbaz, Sib -o- Soran, Mehrestan, and Khaash (Figure 1). [6] It is widely distributed and different parts of *WS* are prescribed, empirically, in traditional medicine, in above locations, for the treatment of diarrhea, vomiting and hypertension. [6-8] Aqueous, ethanol, methanol, petrol, and chloroform extracts of leaves and roots of *WS* have been examined on *S. typhimurium*, *Sh. dysenteriae* and *E. coli*. Nevertheless, the effect of berries of *WS* is ambiguous. [9-11]

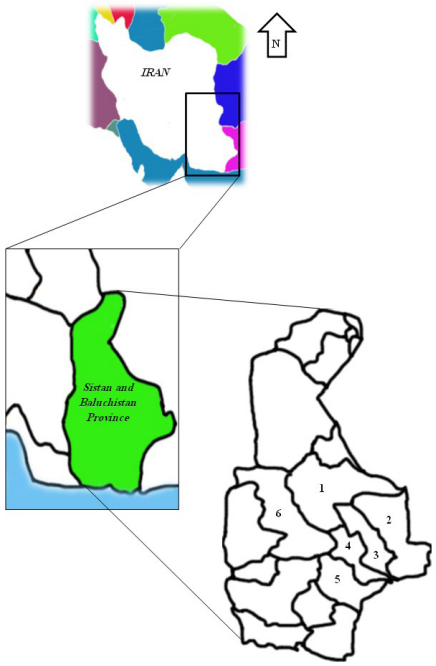

Figure 1. Cities of Sistan and Baluchistan province used for sampling. 1: Khaash, 2: Saravan, 3: Sib -o- Soran, 4: Mehrestan, 5: Sarbaz, 6: Iranshahr

Intestinal bacteria are divided into opportunistic pathogens like *Escherichia coli*, and primary pathogens including *Salmonella* and *Shigella* spp. They infect the digestive system of human and animal, lead to diarrhea and digestive system disorders. [12] In addition, they could act as food borne pathogen via consumption of contaminated water or meat. [13] Antibiotics are used to inhibit the growth of these bacteria and it results in the emergence and development of the resistance isolates. Hence, to discover new agents with distinct origin, as substitution for antibiotics, is logical, nowadays [2].

The present study is carried out to investigate the antimicrobial activity of different dilutions of extract of fruits of *WS*, using a microtitre plate method, against three commercially available bacterial strains, including *E. coli*, *S. typhimurium*, and *Sh. dysenteriae*, as representative of intestinal food borne gram-negative pathogens in Iran.

3. Materials and Methods

3.1 Bacterial Strains and Culture Media

E. coli (ATCC® 25922™; PTCC® 1399™), *S. enterica subsp. enterica* serovar *typhimurium* (ATCC® 14028™; PTCC® 1709™), and *Sh. dysenteriae* (PTCC® 1188™), were delivered from archive of Laboratory of Microbiology, Faculty of Veterinary Medicine (LMFVM), University of Zabol, Zabol, Sistan and Baluchistan, Iran, and used for the current study. Bacterial cultures were grown in Peptone Water Broth (PWB). Two hours culture of tested bacteria, amplified in 5 mL PWB, were adjusted to 0.5 McFarland standard (about 10^8 CFU, confirmed by plate colony count). [2]

3.2 Plant Material

WS were collected from cities of Sistan and Baluchistan province (Figure 1), Iran, at the markets, and at the homes of traditional healers during April - July 2017, and transferred to LMFVM. The identification of the plant was conducted entirely by Department of Plant Pathology, Faculty of Agriculture, University of Zabol, Iran.

3.3 Extract Preparation

Briefly, enzymatic extract of *WS* fruits was obtained by homogenization of 10 g of *WS* berries in 60mL of 85% NaCl for 24 hours via mild shaking, and then, centrifugation at 20,800 × g for 30 min at 4°C. The supernatant was filtered and applied for *invitro* study or stored at 4°C for next steps. [6, 14] Identification and quantification was performed, considering total protein concentration of enzymatic extract via Bradford method. [15]

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3.4 Formulation of Different Concentration of Extract

Five dilutions of extract (0%, 25%, 12.5%, 6.25%, and 3.125%) were prepared in this study.

3.5 Antimicrobial Assay

100 µL of adjusted culture to 0.5 McFarland standard was distributed into flat-bottomed 96-well microtiter plates and mixed with 100 µL of different concentration of *WS* berries extract. As microorganism control, 100 µL of PWB was mixed with 100 µL of adjusted culture to 0.5 McFarland standard. In addition, culture medium control consist of 100 µL of PWB mixed with 100 µL of PWB was included. Plates were incubated at 37 °C, and the growth, as Optical Density (OD), was evaluated using a micro-plate reader (Stat fax-2100, UK), set at 490 nm, at time 0 and 24 hours after incubation. All bioassays were carried out in triplicates. The antimicrobial assay was carried out for *E. coli*, *S. typhimurium* and *Sh. dysenteriae*, individually. [2]

3.6 Statistical Analysis

The growth curve of tested bacterium, based on OD, for different concentration of *WS*, and control, was computed and the slope value was calculated via regression coefficient (B). In better words, the increases and decreases in growth rate were calculated by slope of trend lines during tow measurements (0 and 24) using Microsoft Excel [16]. More negative slopes value demonstrated more inhibition. Statistical Package for the Social Sciences (SPSS) was applied to analyze statistics via ANOVA and to identify differences with p-value <0.05 [16].

4. Results and Discussion

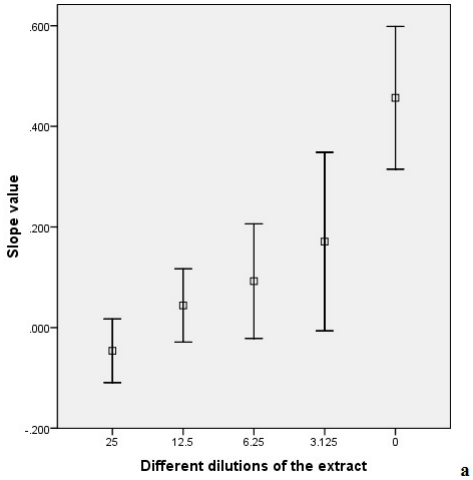
High interest in traditional medicine has been declared by WHO. [3] In Sistan and Baluchistan, one of the biggest provinces of Iran, primary health care have been mediated via traditional medicines. [5] Bacterial infections and inflammation are among the sicknesses treated by traditional healers in study area. Few investigations regarding traditional medicine have been accomplished in South-East of Iran, Sistan and Baluchistan, one of the old provinces of Iran. [2]

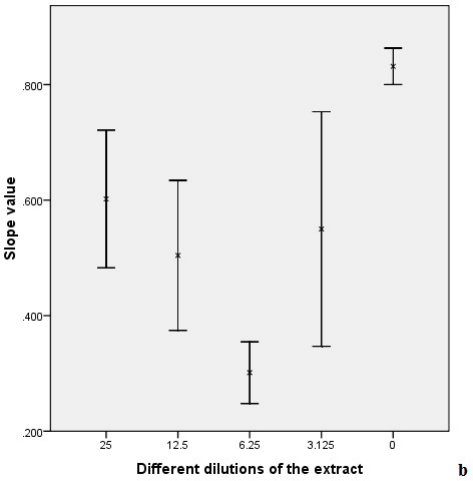
Regional healers for treatment of diarrhea administrate *WS* in Sistan and Baluchistan. Based on our literature review no study about *WS* was performed in the study area [4].

There are literally many published scientific papers from around the globe describing the antimicrobial activities of *WS* extracts and its chemical constituents, but none of them deals with fruit extract of *WS*. [17-19]

As a continuous work to our previous relevant study, [2] the present study is performed to figure out the effect of different concentrations of crude enzymatic extract of *WS* berries against *E. coli*, *S. typhimurium* and *Sh. dysenteriae* growth, and also, to register the local understanding of traditional medicines' use by residents in Sistan and Baluchistan.

The growth rate of *E. coli*, *S. typhimurium* and *Sh. dysenteriae* were assessed via the calculation of slope line trend. All microorganisms tested were found to be susceptive to the extract and their growth was inhibited compared to their control (Figures 2). The lowest growth rate, with significant difference (p<0.05), was observed at 25%, 12.5% and 6.25% of the concentrations of the extract for *E. coli* (Figures 2a). Moreover, based on figure 2b, all concentrations of the extract showed inhibitory effect, with statistical significant difference (p<0.05), on the growth of *S. typhimurium* compared to the microorganism control. Finally, two concentrations of the extract, including 12.5% and 6.25%, decreased the growth rate of *Sh. dysenteriae*, statistically significant (p<0.05), compared to its control (Figure 2c). In addition, as can be seen in figure 2, notably (p<0.05), 12.5% and 6.25% of concentrations of the crude enzymatic extract of *WS* berries showed the best inhibitory effect for three tested bacteria.





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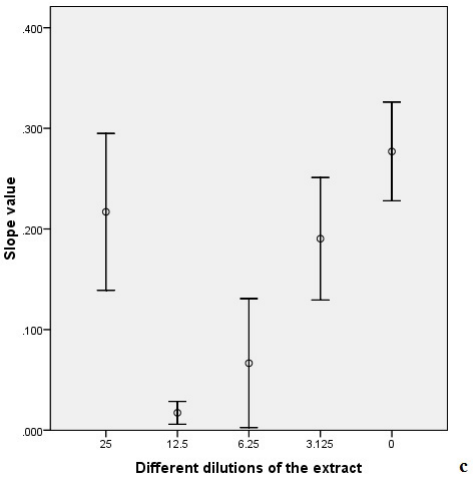

Figure 2. The effect of different dilutions of extract on *E. coli* (a); *S. typhimurium* (b); *Sh. dysenteriae* (c); the dots show slope value of trend lines and error bars indicate 95% confidence of interval of Mean. More negative slopes value demonstrated more inhibition

As a first report, (1) the inhibitory effect of *WS*, particularly crude enzymatic extract of its berries, on *Sh. dysenteriae* is reported, (2) comparison of inhibitory effect of the extract on bacterial growth is performed in Iran by a simple and user-friendly method, (3) the representative of three food borne pathogens were selected, and the most effective concentration of the extract, among examined concentrations is introduced for pathogens, individually.

These results are well comparable to the in vitro antibacterial activities of crude extracts of *WS* against human pathogenic bacteria such as *S. typhimurium* and *E. coli*. [11] The method used in the present work and the component of the plant are different. Furthermore, strain TA100 of *S. typhimurium* and DH5α strain of *E. coli* was investigated by Arora et al. [11]

The present study deals with only crude enzymatic extract of fruit of *WS*. It is suggested to specify and contemplate purifying all the fractions and check the antibacterial activity of individual compounds. Checking the efficiency of individual compounds is more desirable and warranted to increase importance of the present investigation. Antimicrobial properties of a non-toxic glycoprotein (*WS*G) extracted from the root tubers of *WS* is documented. Evidences clearly indicate that *WS*G is a protease inhibitor and exertion of antifungal activity could be due to its protease inhibitory nature. Moreover, findings ruled out the possibility that *WS*G is a ribonuclease/ deoxyribonuclease [10]. This kind of glycoprotein may be found to accomplish the antimicrobial properties of the fruit of *WS* that requires more investigation.

Antimicrobial activity of leaf extract of *WS* against antibiotic resistant *Staphylococcus aureus* was assessed and concluded that ethanol extract of *WS* leaf might be exploited as natural drug for the treatment of several infectious diseases caused by this pathogen [20]. A study evaluated the antibacterial activity of aqueous and alcoholic extracts of root and leaves of *WS* against pathogenic bacteria including *S. typhimurium* ATCC 23564, *E. coli* K-12 DSM 4060 and *Staphylococcus aureus* ATCC 9144 by in vitro agar gel diffusion method and it was found to possess strong antibacterial activity against mentioned bacteria. [21] According to the antimicrobial properties of the plant, our findings in the current study was not different from those of Bokaeian and Saeidi [20] and Owais et al [21].

A study investigated the antibacterial activity of *WS* root (*WS*R) against *E. coli* O78. The turbidity optical density was measured. The results revealed that the maximum inhibition of bacterial growth was observed at 1:8 dilution of *WS*R extract. The authors concluded that *WS*R possessed good antibacterial activity [22]. It is consistent with our findings which was observed the lowest growth rate of *E. coli*, at 25%, 12.5% and 6.25% of concentrations of the extract ,with significant difference ($p<0.05$).

As shown in Figure 2a, the slope values, for the extract with the E.coli showed a dose-dependent decrease with the increase in the concentrations of extract, while, it was not seen for both *S. typhimurium* and *Sh. dysenteriae* (Figures 2b and 2c). One study found that *WS* caused dose-dependent suppression of α2-macroglobulin (an indicator for anti-inflammatory drugs) in the serum of rats inflamed by injection of carrageenan suspension. [23] Furthuremore, a study revealed that the aqueous extract of *WS*R inhibited the growth of bacteria in dose-dependent manner. [21] Also, in present study, as detailed information, the dose-dependent response of the extract of a *WS* component against *E. coli* has been shown.

Due to the antibacterial activity of the extract, the results presented in this paper documented that tested plant used by the healers in Sistan and Baluchistan for treatment of diarrhea, may act toward diarrheal diseases believed to be of bacterial origin. These facts support the medicinal value of *WS* and suggest that it could be the new sources of antibacterial therapies.

Again, it is worthwhile to note that according to our literature review, the present study is the first report related to the effect of different concentrations of enzymatic extract of *WS* fruits on three important intestinal food borne gram-negative pathogens including *E. coli*, *S. typhimurium*, and *Sh. dysenteriae*. Among tested bacteria, different concentrations of *WS*, in term of inhibitory effect, are compared, statistically. All microorganisms tested were found

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to be susceptive to the extract. Different concentration of the extract, showed various extent of growth inhibition among tested bacteria, individually (Fig 3). The power of the inhibition of the extract among three bacteria, based on the concentration is compared in table 1. As can be seen in table 1, notably ($p<0.05$), 25% of concentration of the extract showed the different inhibitory effect among three tested bacteria (table 1, Fig 3), indicating that the inhibitory effect of the 25% of concentration of the extract could be species-specific among bacterial population. The decreasing order of the growth rate, with significant difference, in confronting with the extract were as *E. coli* > *Sh. dysenteriae* > *S. typhimurium* (Fig 3). The results clearly indicated that *E. coli* and *Sh. dysenteriae* are the bacteria with the highest sensitivity to the extract compared to *S. typhimurium* (Fig 3). There are studies that report selective antibacterial activity of extract of *WS* inhibiting the growth of bacteria, which is consistent with our results. [24]

Table1. Comparison of the inhibitory effect of different concentration of the extract on the growth of tested bacteria

tested bacteria	Concentration (%)			
	25	12.5	6.25	3.125
E. coli vs *S. typhimurium*	■	■	■	
E. coli vs *Sh. dysenteriae*	■		■	
S. typhimurium vs *Sh. dysenteriae*	■	■		
E. coli vs *Sh. dysenteriae*	■			
The comparison that are significantly different ($p<0.05$) from each other at the same concentration point indicated by ■.				

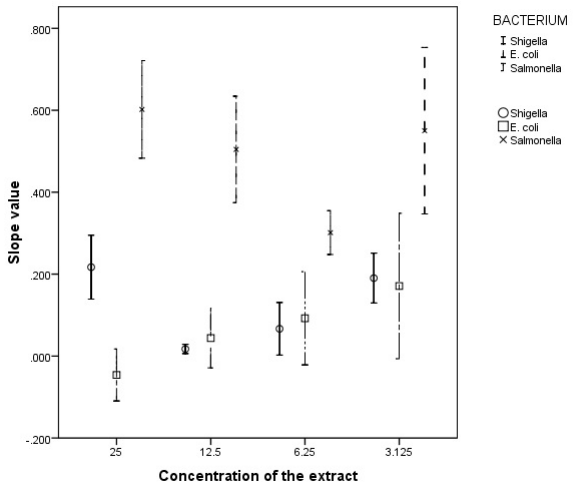

Figure 3. The effect of different dilutions of extract on three tested bacteria; Dots show slope value of trend lines and 95% confidence of interval of Mean are presented by error bars. More negative slopes value demonstrated more inhibition

A study screened the crude extracts of different parts of *WS* including unripen fruit; ripen fruit, and calyx, for their antimicrobial activity invitro against Bacillus subtilis, Pseudomonas aeruginosa, and Enterobactor aerogens by disc diffusion assay. Chloroform extract of calyx of *WS* showed highest activity against *Bacillus subtilis*. [25] Our study was the first report to evaluate the effect of crude fruit extract on *E. coli*, *S. typhimurium* and *Sh. dysenteriae* and the results obtained in the present study may be due to the genera of tested bacteria and methodology compared to the study conducted by Singariya et al. [25]

Antimicrobial activity of crude acetone extract from the aerial part of *WS* was tested in vitro against six pathogenic bacteria, using disk diffusion method, in comparison with gastrointestinal microbiota, and the results suggested that *WS* could act as an effective antibacterial agent against human pathogenic bacteria with lowered harmful effect on bifidobacteria [9]. The examination of different part of *WS* with different methodology in the present work may justify the difference of our observed data compared to the findings of Halamova et al [9].

Identification, extraction, and purification of more than 35 chemical constituents of *WS* has been widely studied. Alkaloids, steroidal lactones, saponins, and withanolides have been introduced as the biologically active chemical compounds of *WS*. [19] The structural and non- structural proteins of examined bacteria, in comparison with other tested bacteria, may play a role to super-induce/prevent the inhibitory effect of the extract [26].

The results of the present study portray the prospect of using *WS* as a substitution for antibiotics in the bacteriology. It is important to demonstrate scientifically that the remedies employed in folk medicine are indeed therapeutically active and therefore, potentially active compounds must be isolated from tested plant and according to our outcomes, *WS* would be interesting candidates for future research regarding to *E. coli*, *S. typhimurium* and *Sh. dysenteriae*. Bear in mind that the mutagenic and/or toxic effects of *WS* is still ill-defined and could act as growth depressor. Further studies on the mutagenesis/toxicity of the plant must be employed, as well as its application in often complex traditional mixtures. It would allow to elucidate possible candidates for future development of antimicrobial agents.

It should not be out of mind that minimum inhibitory concentration, double disc diffusion test, standard susceptibility breakpoints and resistance cut-off breakpoints for this plant fruit extracts need further investigation.

Studies showed that fruits extract of *WS* possesses good radical scavenging activity. [27] It is, also, reported that synthetic oxidants presenting in both food and drugs

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can lead to undesirable health effects. With the latest trend, crude fruit extracts of *WS* as antioxidants have been potentially proposed to add by many food technologists to increase the nutrient values. The use of natural antibacterials for treating diseases, and as food additives, have better consumer acceptability and a trend over the use of the available synthetic products. [27]. Our result, as a research of quantification of the antibacterial activities of the *WS*, indicated that two concentration of the extract, including 12.5% and 6.25%, play significant role to decrease growth rate of three important food borne pathogens, including *E. coli*, *S. typhimurium* and *Sh. dysenteriae*. Our result is important since the information on the antibacterial properties of *WS* against *E. coli*, *S. typhimurium* and *Sh. dysenteriae* is available prior to incorporating them into food products. The findings may be used as a fundament for further experiment in food technologies to control diseases. It is notable that harmful adverse effect on beneficial human microbiota, regarding to plant extracts and compounds, need more investigation. On the other hand, vegetarianism can also lead to increase the demand for substitution of plant material instead of chemical drugs, especially antibiotics. Through this investigation, we have shown that all dilutions of extract exhibit antibacterial activities against three tested bacteria, proposed a good potential to be used in therapeutics. The results presented in this report will also provide a suitable guide in choosing dilutions of extracts by the medical practitioners as natural antibacterial treating and controlling diseases.

In sum, this paper reports and establishes a scientific basis for the therapeutic use of *WS* as an antibacterial agent against three food borne pathogens. This experiment reveals and proposes the effective dilutions of crude enzymatic extract of *WS* berries against the growth of *E. coli* (25%, 12.5%), *S. typhimurium* (25%, 12.5%, 6.25%, 3.125%) and *Sh. dysenteriae* (12.5% and 6.25%), and also, it increases the local understanding of traditional medicines' use by residents in Sistan and Baluchistan. 12.5% and 6.25% dilutions of crude enzymatic extract of fruit of *WS* can be proposed for further research, as food additive to remedy ailments related to examined bacteria.

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Conflict of Interest

The authors declare no conflict of interest for all potential sources of bias, including affiliations, funding sources, and financial or management relationships.

References

[1] Cragg GM, Newman DJ, Snader KM. Natural products in drug discovery and development. J. Nat. Prod., 1997, 60: 52-60.
DOI: 10.1021/np9604893

[2] Shahriari R, Salari S, Shahriari S. In vitro study of concentration-effect and time-course pattern of white alum on *Escherichia coli* O157:H7 growth. Afr. J. Tradit. Complement. Altern. Med., 2017, 14: 311-318.
DOI: 10.21010/ajtcam.v14i2.32

[3] Ekor M. The growing use of herbal medicines: issues relating to adverse reactions, challenges in monitoring safety. Front. Pharmacol., 2013, 4: 177.
DOI: 10.3389/fphar.2013.00177

[4] Afshar (Sistani) I. Sistan Traditional Medicine. Zabol, Zabol University Press, 2003: 45-63. (Text in Persian)

[5] Naseri nia MH, Nazerian NM, Naseri nia I. A survey on variety of common traditional treatments in Sistan and Baluchistan. J. Islam. and Iran. Trad. Med., 2013; 4: 55-66.

[6] Beigomi M, Ghods Rohani M, Mohammadifar MA, Hashemi M, Valizadeh M, Ghanati K. Comparison of textural and sensory characteristics of ultrafiltrated white cheese produced by paneer bad (Withania coagulans) protease and fungal rennet. Iranian Journal of Nutrition Sciences & Food Technology, 2013, 8: 253-262. (Text in Persian)

[7] Hemalatha S, Wahi AK, Singh PN, Chansouria JP. Hypolipidemic activity of aqueous extract of Withania coagulans Dunal in albino rats. Phytother. Res., 2006, 20: 614-617.
DOI: 10.1002/ptr.1916

[8] Jaiswal D, Rai PK, Watal G. Antidiabetic effect of Withania coagulans in experimental rats. Indian J. Clin. Biochem., 2009, 24: 88-93.
DOI: 10.1007/s12291-009-0015-0

[9] Halamova K, Kokoska L, Polesny Z, Macakova K, Flesar J, Rada V. Selective in vitro growth inhibitory effect of *Withania somnifera* on human pathogenic bacteria and bifido bacteria. Pak. J. Bot., 2013, 45: 667-670.

[10] Girish KS, Machiah KD, Ushanandini S, Harish Kumar K, Nagaraju S, Govindappa M. et al. Antimicrobial properties of a non-toxic glycoprotein (*WS*G) from *Withania somnifera* (Ashwagandha). J. Basic

Microbiol., 2006, 46: 365-374.
DOI: 10.1002/jobm.200510108

[11] Arora S, Dhillon S, Rani G, Nagpal A. The in vitro antibacterial/synergistic activities of *Withania somnifera* extracts. Fitoterapia, 2004, 75: 385-388.
DOI: 10.1016/j.fitote.2004.01.002

[12] Quinn PJ, Markey BK, Carter ME, Donnelly WJC, Leonar FC, Hartigan P. et al. Veterinary Microbiology and Microbial Diseases. 2nd ed. USA:Wiley-Blackwell Publication, 2011: 263-287.

[13] Priyanka B, Patil RK, Dwarakanath S. A review on detection methods used for foodborne pathogens. Indian J. Med. Res., 2016, 144: 327-338.
DOI: 10.4103/0971-5916.198677

[14] Chazarra S, Sidrach L, López-Molina D, Rodríguez-López JN. Characterization of the milk-clotting properties of extracts from artichoke (Cynara scolymus, L.) flowers. Int. Dairy J., 2007, 17: 1393-1400.
DOI: 10.1016/j.idairyj.2007.04.010

[15] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical. Biochem., 1976, 72: 248-254.
DOI: 10.1006/abio.1976.9999

[16] Naroyi V, Salari S. Classification of resistance of *Escherichia coli* isolated from poultry with colibacillosis to the complement. Alexandria Journal of Veterinary Sciences, 2017, 54: 13-16.
DOI: 10.5455/ajvs.264500

[17] Mishra LC, Singh BB, Dagenais S. Scientific basis for the therapeutic use of *Withania somnifera* (ashwagandha): a review. Altern. Med. Rev., 2000, 5: 334-346. PMID: 10956379

[18] Bussmann RW, Malca-Garcia G, Glenn A, Sharon D, Chait G, Diaz D. et al. Minimum inhibitory concentrations of medicinal plants used in Northern Peru as antibacterial remedies. J. Ethnopharmacol., 2010, 132: 101-108.
DOI: 10.1016/j.jep.2010.07.048

[19] Kang CG, Hah DS, Kim CH, Kim YH, Kim E, Kim JS. Evaluation of antimicrobial activity of the methanol extracts from 8 traditional medicinal plants. Toxicol. Res., 2011, 27: 31-36.
DOI: 10.5487/tr.2011.27.1.031

[20] Bokaeian M, Saeidi S. Evolution of antimicrobial activity of leaf extract of *Withania somnifera* against antibiotic resistanct *Staphylococcus aureus*. Zahedan J. Res. Med. Sci., 2015, 17: e1016.

[21] Owais M, Sharad KS, Shehbaz A, Saleemuddin M. Antibacterial efficacy of *Withania somnifera* (ashwagandha) an indigenous medicinal plant against experimental murine salmonellosis. Phytomedicine, 2005, 12: 229-235.
DOI: 10.1016/j.phymed.2003.07.012

[22] Kumari M, Gupta RP. In vitro antibacterial effect of *Withania somnifera* root extract on *Escherichia coli*. Vet. World, 2015, 8: 57-60.
DOI: 10.14202/vetworld.2015.57-60

[23] Anbalagan K, Sadique J. Role of prostaglandins in acute phase proteins in inflammation. Biochem. Med., 1984, 31: 236-245. PMID: 6202298

[24] Kamijo M, Kanazawa T, Funaki M, Nishizawa M, Yamagishi T. Effects of Rosa rugosa petals on intestinal bacteria. Biosci. Biotechnol. Biochem. 2008, 72: 773-777.
DOI: 10.1271/bbb.70645

[25] Singariya P, Mourya KK, Kumar P. Antimicrobial activity of the crude extracts of *Withania somnifera* and Cenchrus setigerus In-vitro. Pharmacognosy Journal, 2012; 4: 60-65.
DOI: 10.5530/pj.2012.27.10

[26] Sharma S, Dahanukar S, Karandikar S. Effects of long-term administration of the roots of ashwagandha and shatavari in rats. Indian drugs, 1985, 29: 133-139.

[27] Fatima I, Hussain T, Rafay M, Akram M, Bano S, Shabbir S. Evaluation of antioxidant activity of leaves and fruits extracts of five medicinal plants. Pak. J. Pharm. Sci., 2017, 30: 1625-1628. PMID: 29084682

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