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Synergistic Antimicrobial Effect of Tribulus terrestris and Bitter Almond Extracts

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Abstract

***Background*:** The antimicrobial effects of the extracts of different kinds of plants have been demonstrated in several studies. However, no study has been conducted so far on the synergistic effects of two herbal extracts on their germicidal effects. In this study, in addition to antibacterial effects of the aqueous, methanol or ethanol extracts of *Tribulus terrestris* and bitter almond on some bacteria, the synergistic effects of the extracts of these two plants were also evaluated.

***Materials and Methods*:** In this experimental study, water, methanol and ethanol extracts of seeds were screened against some bacterial strains. Seeds were extracted by percolation method. Aliquots of the extracts at variable concentrations were then incubated with different bacterial strains, and the antimicrobial activities of the extracts from seeds were determined by MIC. Three antibiotics were used as reference compounds for antibacterial activities. Seeds extract inhibited significantly the growth of the tested bacterial strains.

***Results*:** The greatest synergistic effect of *T. terrestris* and bitter almond extracts is detected in methanol and aqueous extracts. Among the bacterial strains tested, *Staphylococcus aureus* was most susceptibility.

***Conclusion*:** The results showed the highest antibacterial effect in the combination of methanol extract of *T. terrestris* and the aqueous extract of the bitter almond.

Introduction

In the past decade interest on the topic of antimicrobial plant extracts has been growing. Use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases [1]. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds [1, 2].

As the center of global health policy and monitoring, in 1978, World Health Organization pointed out in the Almaty Declaration for the first time that a majority of human society still believe in herbal remedies and use them to maintain their public health [1]. For centuries, herbal medicines were the only available source to treat most diseases. In this era, despite the impressive development of chemical medicine, herbal drugs are still being used in large-scale so that in some countries this medicine are an integral part of the drug treatment system and have a more thriving market compared to chemical drugs [2]. Antibiotic resistance is one of the biggest public health problems in the world. Antibiotic resistance is not a new problem. Some resistant strains were appeared a short time after the discovery of antibiotics over 50 years ago. Penicillin and other antibiotics which initially were considered as a miracle drug were later faced with the problem of resistant strains [3]. Hence, nowadays extensive studies are conducted on the new drugs with relatively high microbial effects. Study and use of medicinal plants which were primarily used to treat infections can introduce new sources of antibiotics by itself. *T. terrestris* and bitter almond are two such plants which were used in traditional medicine for treating many infections and there are many papers on these factors and their effects available at present [4]. *T. terrestris* is an herbal medicine that is used in traditional medicine for treating genitourinary tract infection especially gonorrhea, rejection of kidney stones, rheumatic pain reliever, reducing blood pressure and stimulating the liver. Bitter and sweet almond oils which are used locally as emollients and healers in cases such as itching in measles, scarlet fever, and eczema in children, have antibacterial and anti-parasitic effects [5].

Screening of medicinal plants for antimicrobial activities and phytochemicals is important for finding potential new compounds for therapeutic use. This paper reports the results of a survey that was done based on folk uses by traditional practitioners in Iran for antimicrobial activity. Thus, this research project, in addition to their antimicrobial potential, attempts to study the synergistic effects of the extracts of these two drugs on some pathogenic organisms as well.

Materials and Methods

Bacterial strains and culture conditions: In this experimental study, bacteria examined include: *Escherichia coli* PTCC 1533, *Pseudomonas aeroginosa* PTCC 1310, *Staphylococcus aureus* PTCC 1113 and *Salmonella typhi* PTCC 1609. These bacteria have been provided from the Iran microbial collections of Iranian Research Organization for Science and Technology (IROST) in lyophilized form. The bacterial samples were cultured according to standard procedures. Since the number of inoculated bacteria is one of the most important variables that affect the outcome of the study, the density of the inoculated microbial suspension has to be standard.

Preparation of plant extracts: Firstly *T. terrestris* fruits and bitter almonds were collected from the Arak villages, Iran in January 2013. Then, they were chopped into small pieces and were weighed and used after being dried. Four grams of *T. terrestris* powder and 4 g of bitter almond powder were separately poured into 3 distinct beaker and a solvents (methanol 80%; ethanol and water) were added. The soggy plants were transferred into distinct percolators after 2 h. When solvents (methanol 80%; ethanol and water) were added, the percolator valves opened so that the flow rate was 4-6 drops/min. Getting assured of the completion of percolation (approximately 72 h later) the extract was concentrated using rotary evaporator with 90 rotation and 45ºC. Then, it was put in the oven with 40ºC so that the extract was completely dry. Five hundred milligrams of the prepared dry extract was dissolved in 1 mL (5%) of DMSO (Dimethyl sulfoxide, Sigma, USA) solvent (concentration 500 mg/mL) and was stored in -20ºC [4].

Evaluation of the antimicrobial activity of the extracts by MIC method: To determine the MIC (minimum inhibitory concentration) from the dilution of 500 mg/mL, the dilutions of *T. terrestris* and bitter almond extracts were prepared separately. Equal amounts of 500 mg/mL extracts were mixed to study the synergistic effect of *T. terrestris* and bitter almond. Then the next dilutions were prepared in serial order. The bacterial suspension (10^6 bacteria/ mL) was then added to each tube.

All inoculated media were placed in 37ºC temperature for 24 h. After this time all the tubes were examined for turbidity. The first tube observed free of turbidity, i.e. absence of bacterial growth was selected as the MIC. To compare the bactericidal effect of the extracts, the bactericidal effects of some antibiotics on the above-mentioned bacteria was used. These antibiotics include: ciprofloxacin, gentamicin and chloramphenicol (Mast, England). MIC was measured by Micro Dilution Broth method according to NCCLS standards.

Results

The methanol extract of *T. terrestris* had the lowest MIC (62.5 mg/mL) in regard with *S. aureus*, which was significantly, lower than the MIC of aqueous and ethanol extract (125 mg/mL). In the case of *S. typhi*, the ethanol extract of *T. terrestris* was the most effective antimicrobial. Aqueous and ethanol extracts of bitter almond, had the greatest effect on *P. aeruginosa*. Both *T. terrestris* and bitter almond had a synergistic effect in alcoholic and aqueous extracts. The results of the effect of antibiotics on bacteria are shown in table 2.

As can be seen from the results in table 1, the methanol extract of *T. terrestris* is the most effective antimicrobial among the ethanol, aqueous and methanol extracts. The antimicrobial effect of methanol extract of *T. terrestris* is not only detectable against Gram-negative bacteria, but also a similar effect can be seen for Gram-positive bacteria. Among the bitter almond extracts, the aqueous and ethanol extracts had similar antibacterial effects. This effect can be seen especially on *P. aeruginosa*. But the antibacterial effect of methanol extract of bitter almond is lower than the previous two extracts. The synergistic effect was seen particularly in alcoholic extract of *T. terrestris* and aqueous extract of bitter almond. The anti-bacterial process of the combined extracts was also detected specially for *S. aureus* and *S. typhi*.

Table 1. MIC of extracts against different bacterial strains

Extract	S. aureus	P. aeruginosa	S. typhi	E. coli
Aqueous extract of T. terrestris	Over 125	125	125	125
Ethanolic extract of T. terrestris	125	62.5	31.25	62.5
Methanolic extract of T. terrestris	62.5	62.5	62.5	62.5
Aqueous extract of bitter almond	125	31.25	62.5	125
Ethanolic extract of bitter almond	125	31.25	62.5	125
Methanolic extract of bitter almond	125	62.5	62.5	125
Aqueous extract of T. terrestris + Aqueous extract of bitter almond	62.5	> 62.5	62.5	62.5
Ethanolic extract of T. terrestris+ Ethanolic extract of bitter almond	62.5	31.25	31.25	31.25
Methanolic extract of T. terrestris+ Methanolic extract of bitter almond	62.5	31.25	31.25	62.5
Methanolic extract of T. terrestris+ Ethanolic extract of T. terrestris	62.5	31.25	31.25	62.5
Methanolic extract of T. terrestris+ Aqueous extract of bitter almond	15.625	31.25	15.625	31.25

Table 2. MIC of bacteria to antibiotics

Antibiotics (mg/mL)	S. aureus	P. aeruginosa	S. typhi	E. coli
Ciprofloxacin	16	32	8	8
Gentamicin	8	8	8	8
Chloramphenicol	64	>512	64	512

Discussion

In this study, the water and methanol extracts from *T. terrestris* and bitter almond extracts exhibited significant inhibitory effect on bacterial growth. Microorganisms play an important role in human diseases. High death rate caused by these factors has always made people to think about ways of dealing with microorganisms. Chemicals extracted from plants, as antimicrobial compounds, are considered as a replacement for synthetic drugs because they have fewer side effects. The importance of studying medicinal plants have been recognized in Iran as well [6]. Several factors affect antibacterial activity of a plant. Factors such as the amount of plant extract, the method of extraction, and the type of solvent used the type of medium and the material and the concentration of the extract can affect the antimicrobial effects of the plant [7, 8]. Another factor that may affect the antimicrobial effects of the extract of a plant is the extraction method and the solvent used [4]. Extracts which are obtained with different methods and using different solvents from the same plant can indicate different antimicrobial effects on particular forms of microorganisms. Different concentrations of the extract also affect its antimicrobial activity. Previous studies [9, 10] showed that the antimicrobial effects of the plant have altered by changing the concentration of the extract. The media used in the antimicrobial tests also has a great influence on the antimicrobial properties of the extracts. Also, usage of the different organs of a plant affects the antimicrobial effect of the extract. Kudi et al. [11] has well shown the different antimicrobial effects of bark and leaves of some plants.

Lechtenberg and Nahrstedt discovered the structure of the HCN-liberating compound in bitter almonds [12]. Because the compound was isolated from *Prunus amygdalus* (synonym *Prunus dulcis*), it was named amygdalin. Amygdalin has subsequently been found widespread in seeds of other members of the Rosaceae like in apples (Malus spp.), peaches (*Prunus persica*), apricots (*Prunus armeniaca*), black cherries (*Prunus serotina*) and plums (Prunus spp.) [13, 14]. The diglycoside amygdalin was the first member to be isolated of a new class of natural products now known as cyanogenic glucosides. Cyanogenic glycosides are present in more than 2,500 different plant species, including many important crop plants [15]. Upon disruption of plant tissue containing cyanogenic glycosides, these are typically hydrolyzed by β-glucosidases with concomitant release of Glc, an aldehyde or ketone and HCN. This two-component system, of which each of the separate components is chemically inert, provides plants with an immediate chemical defense against attacking herbivores and pathogens [16]. In addition to their possible defense function, accumulation of cyanogenic glucosides in certain angiosperm seeds may provide a storage deposit of reduced nitrogen and sugar for the developing seedlings [17].

The results of this study are in agreement with the study of Al-Bakri et al. [18], they reported the antibacterial activity of bitter apricot extract against several bacterial strains. They supposed that the antibacterial activities might be brought by amygdalin, alkaloids, flavonoids, tannins and phenolic compounds.

As the Yemeni *T. terrestris* had no detectable anti-bacterial activity against any of the reference bacteria [19] and all parts (fruits, stems plus leaves and roots) of Turkish *T. terrestris* showed activity against all reference bacteria [20], but only fruit and leaf of Indian *T. terrestris* were active against exclusively *E. coli* and *S. aureus* [21], it can be argued that antibacterial activity of Iranian *T. terrestris* against the reference bacteria is similar to Turkish *T. terrestris* [22].

The antimicrobial effects of the extracts of different plant have been demonstrated in several studies. However, no study has been conducted so far on the synergistic activity of two herbal extracts on their germicidal effects. In this study, in addition to antibacterial effects of the aqueous, methanolic or ethanolic extracts of *T. terrestris* and bitter almond on some bacteria, the synergistic effects of the extracts of these two plants were also evaluated. The main limitation of this study is to obtain dry extracts were prepared. Among the *T. terrestris* extracts, the methanol extract has the most antimicrobial effect. The methanol extract with an MIC equal to 62.5 mg/mL affect the Gram-negative and Gram-positive bacteria and prevents them from growth and reproduction.

All three extracts of the bitter almond had almost the same effect on the tested bacteria. However, the aqueous and ethanol extracts had a lower MIC in case of *P. aeruginosa*. It can be derived from the results of this study that basically the alcoholic extracts of *T. terrestris* have a greater bactericide effect compared to its aqueous extract; however the alcoholic extracts of the bitter almond, particularly the ethanolic extract have almost the same effect as the alcoholic extract of *T. Terrestris*. A comparison between the bactericide effect of the aqueous and alcoholic extracts of bitter almond leads to similar results. In the synergistic study of the two plants' extracts, the highest antibacterial effect achieved by the combination of methanol extract of *T. terrestris* and aqueous extract of bitter almond. In addition, having a relatively good effect on the Gram-negative bacteria, this combination shows highly effective on Gram-positive bacteria. So that the synergistic effect increases the MIC value up to about 10 folds compared to each extract alone. The greatest antibacterial effect of *T. terrestris* extracts is detected in its methanolic extract. The greatest effect of the bitter almond extracts on the bacteria belongs to its aqueous and ethanol extracts. The greatest synergistic effect of *T. terrestris* and bitter almond extracts is detected in methanol and aqueous extracts. Finally, it is suggested to further the antimicrobial effect of the plant extract the active ingredients are determined.

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Authors' Contributions
All authors declare that they have no conflict of interest.

Conflict of Interest
The authors declare no conflict of interest.

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References

1. Waller DP. Methods in ethnopharmacology. J Ethnopharmacol. 1993; 38(2-3): 189-195. PMID: 8510468
2. Diallo D, Hveem B, Mahmoud MA, et al. An ethnobotanical survey of herbal drugs of Gourma district, Mali. Pharm Biol. 1999; 37(1): 80-91. [Link]
3. Chatterjee SK, Bhattacharjee I, Chandra G. In vitro synergistic effect of doxycycline & ofloxacin in combination with ethanolic leaf extract of Vangueria spinosa against four pathogenic bacteria. Indian J Med Res. 2009; 130(4): 475-478. PMID: 19942754
4. Abtahi H, Ghazavi A, Karimi M, et al. Antimicrobial activities of water and methanol extracts of bitter apricot seeds. J Med Sci. 2008; 8(4): 433-436. doi: 10.3923/jms.2008.433.436
5. Gopinath V, MubarakAli D, Priyadarshini S, et al. Biosynthesis of silver nanoparticles from Tribulus terrestris and its antimicrobial activity: A novel biological approach. Colloids Surf B Biointerfaces. 2012; 96: 69-74. doi: 10.1016/j.colsurfb.2012.03.023
6. Obeidat M. Antimicrobial activity of some medicinal plants against multidrug resistant skin pathogens. J Med Plants Res. 2011; 5(16): 3856-3860. [Link]
7. EL-Kamali HH, EL-Amir MY. Antibacterial activity and phytochemical screening of ethanolic extracts obtained from selected Sudanese medicinal plants. Curr Res J Biol Sci. 2010; 2(2): 143-146. [Link]
8. Vuorela P, Leinonen M, Saikku P, et al. Natural products in the process of finding new drug candidates. Curr Med Chem. 2004; 11(11): 1375-1389. PMID: 15180572
9. Swenson JM, Wong B, Simor AE, et al. Multicenter study to determine disk diffusion and broth microdilution criteria for prediction of high - and low-level mupirocin resistance in Staphylococcus aureus. J Clin Microbiol. 2010; 48(7): 2469-75. doi: 10.1128/JCM.00340-10
10. Dash B, Sultana S, Sultana N. Antibacterial activities of methanol and acetone extracts of fenugreek (Trigonella foenum) and coriander (Coriandrum sativum). Life Sci Med Res. 2011; 27: 11-15. [Link]
11. Kudi AC, Umoh JU, Eduvie LO and Gefu J. Screening of some Nigerian medicinal plants for antibacterial activity. J Ethnopharmacol. 1999; 67(2): 225-228.
12. Lechtenberg M, Nahrstedt A. Cyanogenic glycosides. In: Ikan R. Naturally occurring glycosides. Chichester, UK: John Wiley- Sons; 1999: 147-191.
13. McCarty CD, Leslie JW, Frost HB. Bitterness of kernels of almond x peach hybrids and their parents. Proc Am Soc Hortic Sci.1952; 59: 254-258.
14. Conn EE. Cyanogenic compounds. Annu Rev Plant Physiol. 1980; 31: 433-451. doi: 10.1146/annurev.pp.31.060180.002245
15. Seigler DS, Brinker AM. Characterization of cyanogenic glycosides, cyanolipids, nitroglycosides, organic nitro compounds and nitrile glucosides from plants. In: Dey PM, Harborne JB. Methods in plant biochemistry, alkaloids and sulfur compounds. London: Academic Press; 1993: 51-93.
16. Nielsen KA, Hrmova M, Nielsen JN, et al. Reconstitution of cyanogenesis in barley (Hordeum vulgare L.) and its implications for resistance against the barley powdery mildew fungus. Planta. 2006; 223(5): 1010-1023. PMID: 16307283
17. Selmar D, Lieberei R, Biehl B. Mobilization and utilization of cyanogenic glycosides: The linustatin pathway. Plant Physiol. 1988; 86(3): 711-716. PMID: 16665975
18. Al-Bakri SA, Nima ZA, Jabri RR and Ajeel EA. Antibacterial activity of apricot kernel extract containing amygdalin. Iraq J Sci.2010; 51(4): 571-576. [Link]
19. Ali NA, Jülich WD, Kusnick C, Lindequist U. Screening of Yemeni medicinal plants for antibacterial and cytotoxic activities. J Ethnopharmacol. 2001; 74(2): 173-9.
20. Abbasoglu U, Tosun F. Antimicrobial activity of Tribulus terrestris L. Growing in Turkey. Hacettepe Univ Eczacilik Fakultesi Dergisi. 1994; 14(2): 81-5. PMID: 11167035
21. Williamson EM. Major herbs of Ayurveda. China: Churchill-Livingstone; 2002: 312.
22. Kianbakht S, Jahaniani F. Evaluation of antibacterial activity of Tribulus terrestris L. growing in Iran. Iran J Pharma Ther. 2003; 2(1): 22-24. [Link]

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