

The antibacterial activity of methanolic *Anacyclus pyrethrum* and *Pistacia lentiscus L.* extract on *Escherichia coli*

Noushin Jalayer-Naderi^{1*}, Mohammad Niakan^{2*}, Elham khodadadi³, Maryam Mohamadi-Motlagh³

¹Department of Oral and Maxillofacial Pathology, Faculty of Dentistry, Shahed University, Tehran, Iran

²Department Microbiology, Faculty of Medicine, Shahed University, Tehran, Iran

³Graduate Student, Faculty of Dentistry, Shahed University, Tehran, Iran

Received: November 2015, Accepted: October 2016

ABSTRACT

Background and Objectives: Antibiotic therapy is the main choice in treatment of *Escherichia coli* induced infections. Using herbal medication is an alternative choice in treatment of diseases. The aim of this study was to determine the antibacterial activity of two traditionally used herbs in Iranian medicine, *Anacyclus pyrethrum* and *Pistacia lentiscus L.*, on *Escherichia coli*.

Materials and Methods: The antibacterial effect of methanolic extract of *Anacyclus pyrethrum* and *Pistacia lentiscus L.* were examined in disk diffusion and skipped wells methods by measuring the diameter of inhibition zones around wells containing different concentrations of extracts from (10-1000 mg/ml) using standard broth macrodilution, method the MIC and MBC were defined.

Results: The methanolic extract of *Anacyclus pyrethrum* from 300 to 1000 mg/ml and the methanolic extract of *Pistacia lentiscus L.* from 30 to 1000 mg/ml showed antibacterial activity on *Escherichia coli*. The MIC of *Anacyclus pyrethrum* and *Pistacia lentiscus L.* methanolic based extract were 800 and 1000 mg/ml, respectively. The MBC was achieved at 800 mg/ml for methanolic extract of *Anacyclus pyrethrum* and *Pistacia lentiscus L.*

Conclusion: The methanolic extract of *Anacyclus pyrethrum* and *Pistacia lentiscus L.* have antibacterial effect on *Escherichia coli* bacteria. This activity is dose-dependent.

Keywords: Antibacterial activity, *Anacyclus pyrethrum*, *Pistacia lentiscus L.*, *Escherichia coli*

*Corresponding author: Dr. Noushin Jalayer-Naderi, Department of Oral and Maxillofacial Pathology, Faculty of Dentistry, Shahed University, No.39, Italia street, Vesal avenue, Tehran, Iran.

Tel: +982188959210

Fax: +982188967618

Email: jalayer@shahed.ac.ir

*Corresponding author: Dr. Mohammad Niakan, Department of Microbiology, Faculty of Medicine, Shahed University, Tehran, Iran; (31, Abdolazadeh St. Keshavarze Blvd, P.O.Box 14155-7435, Tehran, 14156, Iran).

Tel: +982889647921

Fax: +982188966310

Email: niakan@shahed.ac.ir

INTRODUCTION

Escherichia coli is a facultative Gram negative bacterium that is generally found in the lower intestine (1). The virulent strains of *E. coli* cause gastroenteritis and infections of urinary tract. In addition to fluid and electrolytes replacement, the antibiotic therapy is the main choice in treatment of diseases caused by this organism (2-3).

Antibiotic therapy is facing with different problems ranging from hypersensitivity to bacterial resistance. Herbal medication is an alternative choice in treatment of different diseases. The native herbal - based medicine are easy available and non-expensive. The effectiveness of some native herbal - based medicine on *E. coli* has been shown in Africa, England and China (4-7).

Anacyclus pyrethrum (locally known as akarkara) and *Pistacia lentiscus L.* (locally named mastaki) are traditionally used herbs in Iranian medicine by physicians such as Avicenna (8). *Anacyclus pyrethrum* (pellitory) from *Anacyclus* genus is a native plant of India and Arabic countries. It has different therapeutic effects such as antimicrobial, analgesic and antioxidant activities (9-10). It has been shown that *Anacyclus pyrethrum* has antimicrobial effects on *Candida albicans*, *Staphylococcus aureus* and strong larvicidal activity against malaria (11-12).

Pistacia lentiscus L. is a native herb of Africa and Mediterranean countries with antimicrobial, antifungal, antioxidant and anti-inflammatory activities (13-14). It has good effect on gastrointestinal diseases due to its anti *Helicobacter pylori* activity (14-15).

The aim of this study was to determine the antibacterial activity of *Anacyclus pyrethrum* and *Pistacia lentiscus L.* on *E. coli*. Based on our knowledge this is the first study on the antibacterial activity of methanolic extract of *Anacyclus pyrethrum* and *Pistacia lentiscus L.* on this organism

MATERIALS AND METHODS

The antibacterial activities of methanolic extracts of *Anacyclus pyrethrum* and *Pistacia lentiscus L.* on *E. coli* were examined using well diffusion method. The *Anacyclus pyrethrum* and *Pistacia lentiscus L.* were purchased from traditional pharmacies in Shiraz, Iran. The samples were verified by the Department

of Pharmacognosy, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Extraction. 200ml of 95% methanol was added to 200g of the chopped, powdered roots of *Anacyclus pyrethrum* and *Pistacia lentiscus L.* in a sterile flask. The mixture left for 24 hours at room temperature and filtered with No.1 filter paper (Whatman Co Germany.) with 150 μ m diameter for liquor filtration. The extract was dried in Water bath at 70 C° for one week. Dried powdered extract was kept at 4°C at tightly closed vial. Different concentrations from 10 mg/ml to 1200 mg/ml in distilled water were prepared from stock solution (16).

Organism. *Escherichia coli* (ATCC 25922) was obtained from the bacterial collection of Department of Microbiology, Medical Faculty, Shahed University.

Disk diffusion test. A swab of bacterial suspension was streaked on Muller Hinton agar plates (Liofilchem Company, Italy).

The columns adjusted to 1.5 \times 10⁸ bacterial/ml and used sterile blank disks of extract dilution (McFarland 0.5 turbidity standard). After overnight 37°C aerobically condition incubation, the zones of inhibition were measured by using an Antibiotic disk Zone Reader after 24 h.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC).

The MIC was measured by broth Macro dilution test (tube Dilution) method updated by Clinical and Laboratory Standards Institute (17). The lowest concentration of extract that inhibited the visible growth of organism was recorded as MIC. 10 μ l of bacterial suspensions with turbidity of McFarland 0.5 were added to test tubes. 1ml of incubated solution with determined MIC were cultured and kept incubated at 37°C for 24 h. The antimicrobial extracts concentration that show inhibition (>MIC) may have killed the bacterium. Subculture plate less than 0.1% of the initial inoculum, first concentration without bacteria growth was considered as the MBC.

RESULTS

Anacyclus pyrethrum. The methanolic extract of

Anacyclus pyrethrum had not inhibitory effect on *E. coli* at 10 to 200 mg/ml concentrations. From 300 to 1000 mg/ml the inhibition zone was noted.

Table 1. shows the inhibition zone diameters in different concentrations of methanolic extract of *Anacyclus pyrethrum* on *E. coli*.

The MIC of methanolic based extract of *Anacyclus pyrethrum* was 800mg/ml (Table 2).

The MBC of methanolic extract of *Anacyclus pyrethrum* was achieved at 900 mg/ml.

Pistacia lentiscus L. The methanolic extract of *Pistacia lentiscus L.* had not inhibitory effect on *E. coli* at 10 to20 mg/ml concentrations. From 30 to 1000 mg/ml the inhibition zone was noted.

Table 1 shows the inhibition zone diameters (mm) in different concentrations of methanolic extract of *Pistacia lentiscus L.* on *E. coli*.

The MIC of methanolic based extract of *Pistacia lentiscus L.* was 1000mg/ml. (Table 2)

The MBC of methanolic extract of *Pistacia lentiscusL.* was at 1000 mg/ml.

DISCUSSION

The study shows methanolic extract of *Anacyclus pyrethrum* and *Pistacia lentiscus L.* have antibacterial effect on *E. coli*. This activity is dose-dependent.

Some previous researches have shown the effectiveness of native derived herbal - based medicine on

E. coli such as the mixture of combining *Agathosma crenulata*, *Dodonaea viscosa* and *Eucalyptus globulus* from Africa and *Potentilla reptans L.* from Anglo-Saxon native herbs (4-5).

Parkia biglobosa(Jacq.) Benth, *Ageratum conyzoides* Linn. From African countries and *M. yunnanensis*, *S. sinensis*, *G. morella*, *E. daneillii*, *M. squamulata*, *S. arborescens* and *B. hancei* from native Chinese herbal medicine have also antibacterial effect on *E. coli* (6-7).

The study shows the methanolic extract of *Anacyclus pyrethrum* from 300 to 1000 mg/ml had inhibitory effect on *E. coli*. Based on our knowledge this is the first study on the antibacterial activity of methanolic extract of *Anacyclus pyrethrum* on *E. coli*.

Selles et al. showed that the essential oil from Algerian *Anacyclus pyrethrum L.* has activity against *Candida albicans* and *Staphylococcus aureus*. Oxygenated sesquiterpenes was the main effective substance (12).

Studies on biologically active substance of *Anacyclus pyrethrum* and its antibacterial effects are very rare. It has not been elucidated that which active component or either biologic mechanism of *Anacyclus pyrethrum* has antibacterial activity on *E. coli*.

The antibacterial activity of *Pistacia lentiscus L.* was studied more than *Anacyclus pyrethrum*. Some studies on this field were focused on oral cavity bacteria (18-20). It has been reported that *Pistacia lentiscus L.* has antibacterial effecton *Staphylococcus aureus*, *Bacillus subtilis*, *Prevotella melaninogenica* and *Klebsiella*

Table1. The inhibition zone diameter of methanolic extract of *Anacyclus Pyrethrum* and *Pistacia lentiscus L.* on *Escherichia coli*

Extract	10 mg/3ml	20 mg/ml	30 mg/ml	40 mg/ml	50 mg/ml	100 mg/ml	300 mg/ml	400 mg/ml	600 mg/ml	800 mg/ml	1000 mg/ml
<i>Anacyclus Pyrethrum</i>	0	0	0	0	0	0	15 mm	15 mm	17 mm	20 mm	23 mm
<i>Pistacia lentiscus L.</i>	0	0	8 mm	12 mm	15 mm	18 mm	19 mm	19 mm	12 mm	15 mm	17 mm

Table 2. Antimicrobial activity of *Anacyclus pyrethrum* and *Pistacia lentiscus L.* methanolic extract on *Escherichia coli* by determining MIC

Extract	10 mg/ml	20 mg/ml	30 mg/ml	40 mg/ml	50 mg/ml	100 mg/ml	300 mg/ml	400 mg/ml	600 mg/ml	800 mg/ml	1000 mg/ml
<i>Anacyclus pyrethrum</i>	+	+	+	+	+	+	+	+	+	MIC-	MIC-
<i>Pistacia lentiscus L.</i>	+	+	+	+	+	+	+	+	+	+	MIC-

pneumoniae (21-24).

The alpha-Pinene, beta-myrcene, beta-pinene, limonene, and beta-caryophyllene are major antibacterial components of essential oil and gum of *Pistacia lentiscus* L. *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis* have different sensitivity to these compounds (21).

It seems that different components of *Pistacia lentiscus* L. have different effects on different bacteria. Mharti et al. demonstrated that germanicol, thunbergol, himachalene, trans-squalene, terpinyl propionate, 3,3-dimethyl and cadina-1,4-diene derived from essential oil of the leaves of *Pistacia lentiscus* L. have antibacterial effect on *Klebsiella pneumoniae*, but not on *Pseudomonas aeruginosa* (23).

The present study shows that methanolic extract of *Anacyclus pyrethrum* and *Pistacia lentiscus* L. have antibacterial effect on *E. coli*. More researches on identifying the effective components of *Anacyclus pyrethrum* and *Pistacia lentiscus* L. on *Escherichia coli* are necessary.

In conclusion, the methanolic extract of *Anacyclus pyrethrum* and *Pistacia lentiscus* L. have antibacterial effect on *Escherichia coli* bacteria. This activity is dose-dependent.

ACKNOWLEDGEMENT

The study was completed by financial support from deputy of research, Shahed University by grant of Drs. Noushin Jalayer Naderi.

REFERENCES

- Singleton P (1999). Bacteria in Biology, Biotechnology and Medicine. Wiley, Hoboken. pp. 444-454.
- Abduzaimovic A , Aljicevic M , Rebic V , Vranic SM, Abduzaimovic K , Sestic S . Antibiotic resistance in urinary isolates of *Escherichia coli*. *Mater Sociomed* 2016;28 :416-419.
- Brooks GF, Carroll KC , Butel JS, Morse SA, Mietzner TA (2010). In: Jawetz, Melnick, & Adelberg's Medical Microbiology. New York: McGraw-Hill, pp. 217-218, 354.
- Zonyane S, Van Vuuren SF, Makunga NP. Antimicrobial interactions of Khoi-San poly-herbal remedies with emphasis on the combination; *Agathosma crenulata*, *Dodonaea viscosa* and *Eucalyptus globulus*. *J Ethnopharmacol* 2013; 148:144-151.
- Watkins F, Pendry B, Sanchez-Medina A, Corcoran O. Antimicrobial assays of three native British plants used in Anglo-Saxon medicine for wound healing formulations in 10th century England. *J Ethnopharmacol* 2012; 144:408-415.
- Adetutu A, Morgan WA, Corcoran O, Chimezie F. Antibacterial activity and in vitro cytotoxicity of extracts and fractions of *Parkia biglobosa* (Jacq.) Benth. stem bark and *Ageratum conyzoides* Linn. leaves. *Environ Toxicol Pharmacol* 2012; 34:478-483.
- Zuo GY, Zhang XJ, Yang CX, Han J, Wang GC, Bian ZQ. Evaluation of traditional Chinese medicinal plants for anti-MRSA activity with reference to the treatment record of infectious diseases. *Molecules* 2012; 17:2955-2967.
- Sharafkandi A (Abu Ali Sina) (2004). *Ganon* 5th ed. Soroush, Tehran. pp. 338-358. (In Persian)
- Usmani A, Khushtar M, Muhammad Arif, Siddiqui M.A, Prakash Sing S, Mujahid M. Pharmacognostic and phytopharmacology study of *Anacyclus pyrethrum*: An insight. *J App Pharm Sci* 2016; 6:144-150.
- Sujith K, Ronald Darwin C, Suba V. Antioxidant activity of ethanolic root extract of *Anacyclus pyrethrum*. *Int Res J Pharm* 2011;2:222-226.
- Pandey V, Agrawal V, Raghavendra K, Dash AP. Strong larvicidal activity of three species of *Spilanthes* (Akarkara) against malaria (*Anopheles stephensi* Liston, *Anopheles culicifacies*, species C) and filaria vector (*Culex quinquefasciatus* Say). *Parasitol Res* 2007; 102: 171-174.
- Selles C, Dib Mel A, Djabou N, Beddou F, Muselli A, Tabti B, et al. Antimicrobial activity and evolution of the composition of essential oil from Algerian *Anacyclus pyrethrum* L. through the vegetative cycle. *Nat Prod Res* 2013; 27:2231-2234.
- Zargari A. Medicinal plants. 4th ed. Tehran: Tehran University Publications; 1985:569-71. (In Persian)
- Paraschos S, Mitakou S, Skaltsounis AL Chios gum mastic: A review of its biological activities. *Curr Med Chem* 2012; 19(14):2292-302.
- Dabos KJ, Sfika E, Vlatta LJ, Giannikopoulos G. The effect of mastic gum on *Helicobacter pylori*: a randomized pilot study. *Phytomedicine* 2010; 17(3-4):296-9.
- Mahon CR, Lehman C L, Manuseles G .The text book of Diagnostic Microbiology .5thed, Elsevier ; 2015:274-312.
- Clinical and Laboratory Standards Institute (2012). (CLSI). M100-S22. Performance standards for antimicrobial susceptibility testing. CLSI, 22rd informational supplement, Wayne, PA, USA.
- Jalayer Naderi N, Niakan M, Mohamadi Motlagh M. Determination of antibacterial activity of *Pistacia len-*

- tiscus* methanolic extract on *Staphylococcus aureus*, *Streptococcus mutans*, *Streptococcus sanguis*, *Pseudomonas aeruginosa*. *JIUMS* 2015; 22 : 67-74. (In Persian)
19. Takahashi K, Fukazawa M, Motohira H, Ochiai K, Nishikawa H, Miyata T. A pilot study on antiplaque effects of mastic chewing gum in the oral cavity. *J Periodontol* 2003;74:501-505.
 20. Aksoy A, Duran N, Koksall F. In vitro and in vivo antimicrobial effects of mastic chewing gum against *Streptococcus mutans* and mutans streptococci. *Arch Oral Biol* 2006; 51:476-481.
 21. Koutsoudaki C , Krsek M, Rodger A. Chemical composition and antibacterial activity of the essential oil and the gum of Pistacial entiscus Var. chia. *J Agric Food Chem* 2005; 53(20):7681-5.
 22. Sakagami H, Kishino K, Kobayashi M, Hashimoto K, Iida S, Shimetani A, et al. Selective antibacterial and apoptosis-modulating activities of mastic. *In Vivo* 2009; 23:215-223.
 23. Mharti FZ , Lyoussi B, Abdellaoui A. Antibacterial activity of the essential oils of *Pistacia lentiscus* used in Moroccan folkloric medicine. *Nat Prod Commun* 2011; 6:1505-1506.