

Research Article

In vivo effect of pimpinella anisum seeds essential oils on Mouse mice BAL b/c breed infected with Listeriosis disease

Amin Hashempour*¹, Mohammad Hosein Sadeghi Zali², Saber Yousefi^{3, 1}, Nazereh Hashempour¹

¹Young Researcher and Elites club, Islamic Azad University, Urmia Branch, Urmia Iran

²Department of Bacteriology, Veterinary Faculty, Islamic Azad University, Urmia Branch, Urmia, Iran

³Department of Microbiology, Virology and Immunology, Urmia University of Medical Sciences, Urmia, Iran

***Corresponding author**

Amin Hashempour

Email: doctor_amin_dvm@yahoo.com

Abstract: Pimpinella anisum L. (Apiaceae) is an annual herb indigenous to the near east and widely cultivated in the Mediterranean regions as well as Mexico and Chile. It has been used as an aromatic herb and spice since Egyptian times. This research was done on antibacterial activity of anise seeds against listeriosis in mouse mice BAL b/c breeds. In this study we injected bacteria to intraperitoneal area of mice's. After 3 day agglutination tests and injection with anise seed essential oils were done. An agglutination test after one week for ensures that the treatment was done. The results were shown that essential oil has 24 percentage improvements. Anise seed effects can use with other products for example ethanol extract, water extract and etc.

Keywords: In vivo, Pimpinella anisum, essential oil, mice BAL b/c, Listeriosis.

INTRODUCTION

In recent years much attention has been devoted to natural antioxidant and their association with health benefits[1-2]. Plants are a large source of new bioactive molecules with therapeutic potentials. Only a small percentage of living plants on Earth have been phytochemically investigated. Plants are thus an enormous reservoir of pharmaceutically valuable molecules to be discovered[3-5].

Pimpinella anisum L. (Apiaceae) is an annual herb indigenous to the near east and widely cultivated in the Mediterranean regions as well as Mexico and Chile. It has been used as an aromatic herb and spice since Egyptian times [3]. The star anise and anise have both been widely used in Iranian traditional medicine for their antimicrobial effects[6]. The antimicrobial properties of these species have been reviewed by several researchers: anise [3,7] and star anise [8-9].

Anise seeds are used as analgesic in migraine and also as carminative, aromatic, disinfectant, and diuretic in traditional medicine [10]. Aniseed has warm and dry nature and can increase milk production, menstruation, urine, and sweat secretion and also making good complexion. It is also effective in polishing of teeth. In some traditional texts, anise is mentioned for melancholy, nightmare, and also in treatment of epilepsy and seizure[11-12].

Aniseed contains 1.5–6.0 mass % of a volatile oil consisting primarily of trans-anethole and also as much as 8–11 mass % of lipids rich in fatty acids, such as palmitic and oleic acids, as well as approximately 4 mass % of carbohydrates, and 18 mass % of protein[13]. Other studies have demonstrated the presence of eugenol trans-anethole, methylchavicol, anisaldehyde, estragole, coumarins, scopoletin, umbelliferone, estrols, terpene hydrocarbons, polyenes, and polyacetylenes as the major compounds of the essential oil of anise seed [7]. Study of the essential oil of Pimpinella anisum L. fruits by GC and GC-MS showed the presence of trans-anethole (93.9%) and estragole (2.4%).

Other compounds that were found with concentration higher than 0.06% were (E)-methyleugenol, α -cuparene, α -himachalene, β -bisabolene, p-anisaldehyde, and cis-anethole [14]. In another study for determination of the composition of essential oil of Pimpinella anisum L. fruits obtained from different geographical areas of Europe, in addition to the major components (trans-anethole (76.9–93.7%) and γ -himachalene (0.4–8.2%)), some other compounds such as trans-pseudoisoeugenyl 2-methylbutyrate, p-anisaldehyde, and methylchavicol were also identified in essential oil[15].

Essential oil and methanol extract of these plants exhibited antibacterial activity against most

tested pathogens, and the maximum effect was observed against *Staphylococcus aureus*, *Bacillus cereus*, and *Proteus vulgaris*. However, combination of essential oil and methanol extracts of these plants showed an additive effect against most tested bacteria especially *Pseudomonas aeruginosa* [16]. The antibacterial potential of aqueous decoctions of black pepper, bay leaf, aniseed, and coriander against 176 bacterial isolates belonging to 12 different genera were detected by the mean of disc diffusion technique. The findings showed that the aqueous decoction of black pepper was the most bacterial-toxic exhibited 75% antibacterial activity and decoction of aniseed exhibited 18.1% antibacterial activity (maximum antibacterial activities exhibited against *Micrococcus roseus*)[17]. In addition to antibacterial activity, the essential oil of aniseed showed significant inhibitory activity against fungi, and the most active component of it was anethol [18].

METHOD AND MATERIALS

Collection of plant material

Pimpinella anisum seeds were collected from around Urmia city west Azarbayjan Iran on September 2013.

Culture and Maintenance of microorganisms

Bacteria were prepared from Iranian Research Organization for Science and Technology (IROST). After preparation this agent was sub cultured. *Listeria monocytogenes* was cultured on blood Agar.

Table 1: characteristics of Bacteria

S. No	Name	Type	PTCC No
1	<i>Listeria monocytogenes</i>	Gram Positive bacteria	PTCC1298

Preparation of plant essential oils:

After collecting plants peeling and dry was done. After drying, essential oil with Clevenger apparatus method was done. 100gram from dry mater added to 400cc sterile water and boiled on heater for 4 hours.

Preparation of mice's:

128 Mice's were prepared from research center of Zist Faravarde pars in Rasht city Guilan. Mice's were transferred to Islamic Azad university Urmia branch's animal houses.

Bacteria injection:

After sub culture, bacteria were transferred to pepton water media. After 24 hours incubation, intraperitoneal injection in mice's was done.

Agglutination test after bacteria injection:

Listeria Agglutination kits were prepared from research center of Zist Faravarde pars in Rasht city Guilan. 3 day after injection bacteria this test was done.

Injection with anise seed essential oil:

After doing Agglutination test, instead of water Injection with anise seeds essential oil for 1 week.

Agglutination test after treatment:

After 1 week Injection with anise seed essential oil, agglutination test for ensures that the treatment was done.

RESULTS

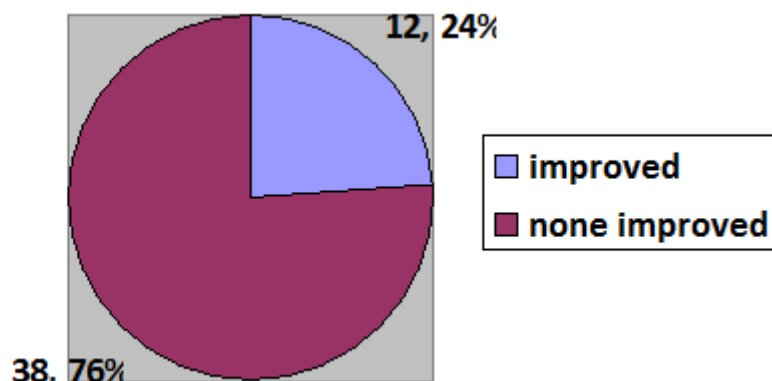
From 128 mice infected with *listeria monocytogenes* 50 mices treatment with essential oils of anise seeds. After 1 week treatment with essential oil results described on table 2. From 50 mice 12 number of mice recovered. 24 percent of mice are improved with essential oil injection (Table-3).

Table 2: agglutination test before and after 1 week treatment with injection of essential oil of anise seeds

no	before	after	no	before	after	no	before	after
1	+	+	18	+	-	35	+	-
2	+	+	19	+	+	36	+	+
3	+	+	20	+	-	37	+	+
4	+	-	21	+	+	38	+	+
5	+	+	22	+	+	39	+	+
6	+	+	23	+	+	40	+	+
7	+	+	24	+	+	41	+	+
8	+	+	25	+	+	42	+	-
9	+	+	26	+	+	43	+	+
10	+	-	27	+	+	44	+	+
11	+	+	28	+	+	45	+	+
12	+	+	29	+	+	46	+	+
13	+	+	30	+	+	47	+	+
14	+	+	31	+	+	48	+	+
15	+	-	32	+	-	49	+	-
16	+	+	33	+	-	50	+	-
17	+	+	34	+	-			

Table 3: number of mice is improved and none improved with essential oil injection

Total number	improved	none improved
50	12	38

**Fig-1: Number of mice is improved and none improved with essential oil injection**

DISCUSSION

Anise (*Pimpinella anisum* L.), is an annual important spice and medicinal plant belonging to the family of Apiaceae, and native to Mediterranean region. Today, anise seeds are an important natural raw material which is used for pharmaceuticals, perfumery, food and cosmetic industries [19].

In this study *in vivo* antibacterial effect of anise seeds essential oil was 24 percent. In many study antibacterial effect of anise seed was shown. For example in 2008 F. A. Al-Bayati with article Synergistic antibacterial activity between *Thymus vulgaris* and *Pimpinella anisum* essential oils and methanol extracts shown antibacterial activity of *Pimpinella anisum* [16]. In another research Faramarz Fekri Yazdi et al showed **Anise seed (*Pimpinella anisum* L.) as an alternative to antibiotic growth promoters on performance, carcass traits and immune responses in broiler chicks** confirmed the antibacterial effect and strengthening **immune responses** of *Pimpinella anisum* [20].

Antimicrobial effects of water and ethanolic extracts of aniseed were studied by Gulcin et al. against 10 bacterial species and also *Candida albicans* with disc diffusion method. In this study, ethanolic extract showed significant inhibitory activity against all tested bacteria but not effective on *Candida albicans*. However, the antimicrobial effect of water extract was not detected against Gram-negative bacteria, *Pseudomonas aeruginosa*, and *Escherichia coli*, but it was effective against *Candida albicans* [7].

The alcoholic extracts of *Pimpinella anisum* seeds also showed antibacterial activity against *Micrococcus luteus* and *Mycobacterium smegmatis* [21].

The antibacterial activities of the aqueous, 50% (v/v) methanol, acetone and petroleum ether extracts of *Pimpinella anisum* L. fruits were tested against 4 pathogenic bacteria (*Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, and *Klebsiella pneumoniae*) by disc diffusion method. The results showed that only aqueous and methanol extracts exhibited fair antibacterial activity against all of the test bacteria and the aqueous extract was found to be more effective than methanolic extract, whereas acetone and petroleum ether extracts cannot inhibit the growth of the pathogenic test bacteria [23].

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