Journal of
Data Acquisition and Processing
ISSN: 1004-9037
https://sjcjycl.cn/
DOI: 10.5281/zenodo.778153

PHYTOCHEMICAL EXAMINATION AND DISINFECTANT ACTIONS OF NOVEL HERBAL FORMULATIONS

Shri Devi. S.D.K
Department of Botany, Sri Sarada College for Women (Autonomous), Salem, Tamilnadu.

A Jansy Isabella Rani
Department of Biochemistry, Vellalar college for women (Autonomous), Erode, Tamilnadu.

G. Sangeetha Devi
Department of Biochemistry, Vellalar college for women (Autonomous), Erode, Tamilnadu

G. Jahirhussain
Department of Botany, Government Arts College (Autonomous), Karur, Tamil Nadu.

Vishwanatha H N
Phytotron Agro Products (India) Pvt. Ltd, Yelahanka, Bangalore (North). Karnataka.

Sajith. S
Department of Chemistry, Government Polytechnic College, Attingal, Thiruvananthapuram, Kerala.

Abstract

Antibiotic-resistant bacteria are difficult to cure, but there may be new hope in the form of medicines that can be extracted from medicinal plants. The researchers set out to determine if essential oil from Pimpinella anisum could inhibit the growth of Pseudomonas aeruginosa and Bacillus subtilis, two common types of bacteria. Chemical composition was determined using gas chromatography mass spectrometry. Agar disk and agar well diffusion methods were used as a preliminary screen for the essential oil's antibacterial capabilities. To find the MIC, a Macrobroth tube test was used. The results showed that Trans-anethole made up the majority of the substances in P. anisum essential oil (89.7%), and that P. anisum essential oil at doses of 0.003 and 0.007 g/ml inhibited the growth of P. aeruginosa and B. subtilis, respectively. The findings demonstrate, then, that the ethnomedical plant has antibacterial properties against the two microorganisms studied. The findings support the idea that the plant's essential oil has therapeutic and/or preservative potential. Further in vivo research and clinical trials are required for justification. The possibility of it being used as an antibacterial agent in topical or oral treatments also needs more study. Future research will focus on the fractionation and characterisation of active compounds.

Keywords: Pimpinella anisum, Essential oil, Chemical composition, Antibacterial effect.

Introduction

The basic foundation for treating microbial (bacterial and fungal) infections is antibiotics. The medical community assumed that with the discovery of these antibiotics and their subsequent usage as chemotherapeutic agents, infectious illnesses would be eradicated forever. However, antibiotic misuse is now the primary driver of the development and spread of resistance strains of many different types of microorganisms1. The widespread application of commercial antimicrobial medications currently used to treat infectious diseases2 has led to the emergence of multiple drug resistance. Herbs and spices are a treasure trove of diverse uses in the kitchen, the bathroom, the pharmacy, and the treatment room. The therapeutic characteristics of these plants make them effective in treating and preventing disease3. Some medicinal herbs employed in traditional Iranian medicine are successful in treating numerous diseases caused by bacterial and oxidative stress [1-10].

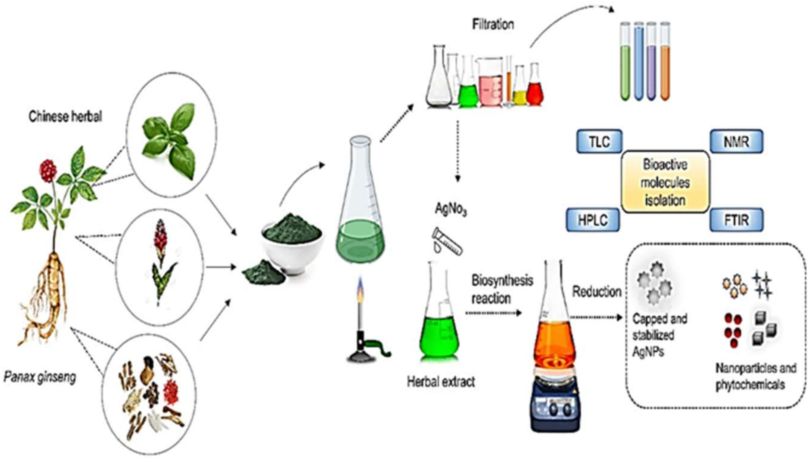

Figure 1: Phytochemical Examination and Disinfectant Actions of Novel Herbal Formulations

Compounds generated during the secondary metabolism of several plants have been exploited for their antibacterial properties5. vitamins (A, C, E, and K), carotenoids, terpenoids, flavonoids, polyphenols, alkaloids, tannins, saponins, pigments, enzymes, minerals, and so on all fall under the umbrella of phytochemicals, which have antibacterial and antioxidant activity6. However, several studies have revealed that phytochemicals have a role in the dynamic between plants and pests or diseases, even if their precise role is yet unknown. New and improved methods have piqued the interest of plant scientists in phytochemical investigations. The pharmaceutical industry's quest for new raw material was greatly aided by these methods. Essential oil is a concentrated hydrophobic liquid containing volatile fragrance components from plants. Essential oils are what give aromatic and therapeutic plants their scent and biological activity. The major components of essential oils are mono- and sesquiterpenes, which include carbohydrates, phenols, alcohols, ethers, aldehydes, and ketones. Spices and herbs have been used as flavoring agents and preservatives8 in cooking since ancient times because of their antimicrobial, antifungal, and antibacterial characteristics. Plants and their essential oils are potentially useful sources of antibacterial chemicals.

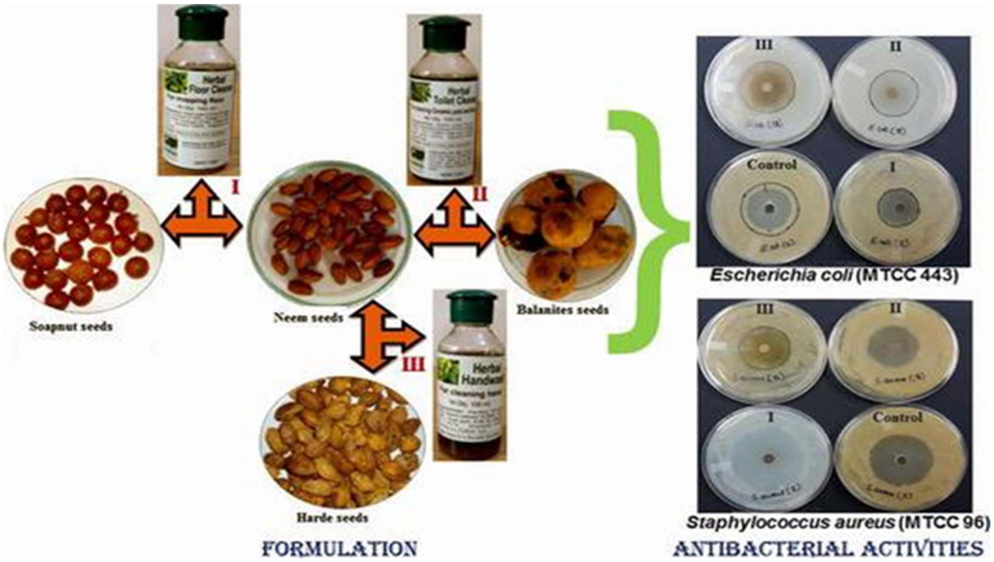

Figure 2: Disinfectant Actions of Novel Herbal Formulations

Therefore, antimicrobial drug discovery can begin with screening essential oils and phytochemicals from plants for their antibacterial properties. A variety of Gram-negative and Gram-positive bacteria, including Staphylococcus aureus, Bacillus subtilis, and Escherichia coli O157:H79, are susceptible to essential oils' antimicrobial effects. Anise (Pimpinella anisum) is a member of the Umbelliferae family and one of the earliest used medicinally. White flowers top this annual grassy plant that grows in the Eastern Mediterranean, West Asia, the Middle East, Mexico, Egypt, and Spain. Its seeds are small and green to yellow in color. The fruits of this plant (aniseeds) are the primary reason it is cultivated. It shares a taste with other spices including anise, fennel, and licorice. Multiple therapeutic benefits, including those on the nervous system, the digestive system, gynecology, fungal illness, and breathing problems, have been attributed to P. anisum10. The main substance discovered in P. anisum essential oil is Trans-anethole. The purpose of this research was to examine the essential oil of the plant for its potential to kill germs in a lab setting [11-15].

Materials and Methods

Gathering of Plant Samples

Kermanshah medicinal plant was harvested for the experimental study. All foreign matter, plant matter, dust, and the like were removed from the sample.

Extraction of Essential Oils Essential oil from fresh, clean, weighted aerial portion P. anisum fruits extracted by hydro-steam distillation using the Clevenger equipment were collected and preserved in sterile vials. To extract the oil, 100–150 g of plant material was placed in a distillation flask (1L), which was then linked via a glass tube to a steam generator and a condenser. A funnel was used to retrieve this. Essential oil molecules were liberated from the plant matter, turning into vapor as the water was heated. The essential oil was extracted from the plant material by heating it in steam but not to the point of burning. Then, the steam containing the essential oil was cooled using a refrigeration system. Three hours were spent using the steam. Essential oil was extracted from the recovered mixture after it had settled. The obtained essential oil was concentrated by filtering the supernatant through anhydrous Na2SO4. After that, the oil was stored in airtight containers in the fridge. The essential oil was diluted multiple times with dimethyl sulfoxide (DMSO) for the antibacterial activity test.

"GC/MS" stands for "gas chromatography mass spectrometry."

P. anisum essential oil was analysed using GC/MS (Shimadzu capillary GC-quadrupole MS system QP 5000) with two fused silica capillary column DB-5 (30 µm, 0.25 mm i.d, film thickness 0.25 µm) and a flame ionization detector (FID) which was operated in EI mode at 70 eV. We used 220 degrees Celsius for the injector and 250 degrees Celsius for the detector. We injected 1 L of each solution in hexane and examined them by heating the column from 60 C to 300 C at a rate of 3 C per minute. The carrier gas used was helium at a rate of 1 ml/min. Peak area as a percentage of total peak area is a convenient way to express the concentration of distinct components within an essential oil. In order to qualitatively identify the various components, their retention durations and mass spectra were compared to those of known reference compounds.

Origin of microbes

Two bacterial species namely P. aeruginosa (PTCC No. 1707) and B. subtilis (ATCC No. 21332) were purchased from Iranian Research Organization for Science and Technology as lyophilized. The bacteria were cultured in Tryptic Soy broth at a steady 37 degrees Celsius for 18 hours. After incubating the broth at 37°C for 24 hours, 60 l of it was plated onto Nutrient agar, and the cell concentration was adjusted with Muller Hinton broth to reach a final concentration of 108 cfu/ml.

Media of culture

Oxoid, UK) instructions, autoclaved, and distributed at 20 ml per plate in 12 × 12cm Petri dishes, as Mueller-Hinton agar is a microbiological growth medium often used for antibiotic susceptibility testing. The plates were incubated overnight to guarantee their sterility.

Assessment of Antimicrobial Performance

Standard screening assays, including agar disk diffusion and agar well diffusion, were utilized to determine whether or not the essential oil of P. anisum possessed antibacterial properties. The essential oil solution was diluted (v/v) six times from a concentration of 1g/ml. Each disk and well received 60 l of the appropriate dilution. After a period of 24 hours' incubation, the widths of growth inhibition zones around the disks and wells were measured. Negative controls consisted of DMSO, whereas positive controls included kanamycin and cephalexin for E. coli and S. aureus, respectively.

The term "minimum inhibitory concentration" (MIC) refers to the lowest concentration of a likely antimicrobial agent that inhibits bacterial growth. The minimum concentration (MIC) is defined as the concentration below which no visible microbial growth occurs. The MBC of a given chemical is defined as the lowest concentration at which all test microorganisms are killed. The final result can be seen by placing 60 l of MIC tube content together with six dilutions on an agar plate and observing the growth. After an incubation period, the smallest concentration at which growth stops is indicative of minimum viable concentration (MBC). The micro-inhibitory concentration (MIC) was calculated using the macrobroth dilution technique. The data were interpreted in accordance with letter, which is widely used in the United States [16-21].

Results

Analytical chemistry

Composition of the plant in RI (Retention Index) including α-pinene (0.1%), sabinene (0.01%), myrcene (0.01), α-phellandrene (0.01%), p-cymene (0.1%), limonene (0.8%), 1,8-cineole (0.1%), cis-b-ocimene (0.01%), fenchone (4.62%), camphor (0.23%), methyl chavicol (2.15%), endo-fenchyl acetate (0.1%), cis- anethole (0.43%), p-anisaldehyde (0.41%), trans-anethole (89.7%), respectively. According to the findings, trans-anethole is the most common component of P. anisum essential oil. On the other hand, sabinene, myrcene, -phellandrene, and cis-b-ocimene are the least common.

Agar-disk-based microplate assay

Essential oil from P. anisum was effective against P. aeruginosa and B. subtilis. The bacteria P. aeruginosa and B. subtilis showed the greatest sensitivity to P. anisum by producing a halo with a diameter of 19 mm at a concentration of 0.031 g/ml. At a dilution of 0.002 g/ml, an inhibitory zone formed around P. aeruginosa but not around B. subtilis. There was no DMSO-induced dead zone detected. Table 1 shows the ranges of dilutions that impede growth.

Essential oil from P. anisum showed the largest diffusion band at 0.031 g/ml, caused by P. aeruginosa and B. subtilis (16 mm). Both bacteria showed no growth inhibition at concentrations of 0.001 g/ml or lower. Table 2 displays the information.

Identifying MIC and MBC

The values of MIC are 0.007 g/ml and 0.003 for B. subtilis and P. aeruginosa, respectively. Table 3 shows that the minimal bactericidal concentration (MBC) for both B. subtilis and P. aeruginosa is 0.015 g/ml. The essential oil from P. anisum has been shown to inhibit the development of P. aeruginosa and B. subtilis, as shown in the table. Not only that, but by upping the dose of the inhibitory zone grew when P. anisum essential oil was added (p0.001). The results showed that the sensitivity of the microorganisms tested to the essential oil varied significantly (p0.001).

Discussion

Although there is growing interest in discovering new drugs through molecular modeling, combinatorial chemistry, and other forms of synthetic chemistry, plant-derived chemicals continue to show promise as a vital source of human therapeutics. Recent accounts detail the significance and use of plants in contemporary drug research. Food preservation, pharmaceuticals, alternative medicine, and natural therapies all use essential oils from plants, which have been used for thousands of years. Essential oils are potential sources of new antibacterial chemicals, notably against bacterial pathogens. Essential oils had varying degrees of success in inhibiting bacterial growth in in vitro investigations included in the work. Many essential oils' antibacterial properties have been studied and rated as either moderate or weak18. P. anisum is an annual member of the Apiaceae family that is utilized as a natural raw material in the pharmaceutical, perfume, culinary, and cosmetics industries, among others19. P. anisum is cultivated largely for its fruit, which is collected as aniseeds in the months of August and September. Aniseeds are used as a flavoring agent, digestive aid, carminative, and to calm stomach cramps because of the 1.5-5% essential oil they contain. P. anisum is one of the medicinal plants used in many treatments in Iran's traditional medicine.

There have been a number of studies conducted on P. anisum extracts and essential oil to determine its chemical composition and pharmacological activities, including its antimicrobial, antifungal, and antibacterial effects. Aniseed's essential oil has antispasmodic, secretolytic, secretomotor, and antimicrobial actions, which explain why it is used medicinally. P. anisum extracts and oil, as well as some oil components, showed potent growth-inhibiting activity in vitro against a wide range of bacteria and fungi that are harmful to humans and other animals. Using high heat can hasten the breakdown of beneficial herbal compounds, thus it's important to follow protocol while working with essential oils. compounds representing 98.78% of the total essential oil composition of P. anisum were identified using mass gas-chromatograph, these compounds including α-pinene, sabinene, myrcene, α- phellandrene, p-cymene, limonene, 1,8-cineole, cis-b- ocimene, fenchone, camphor, methyl chavicol, endofenchyl acetate, cis-anethole, p-anisaldehyde and trans-anethole [22-23].

Trans-anethole made up 89.7 percent of the total compounds in the essential oil of P. anisum. Alkyl alkyl phenol ethers include trans- anethole. Trans-anethole exists as both a cis and a trans isomer, with the trans isomer always predominating in nature. P. anisum essential oils, as well as star P. anisum essential oils, contain anethole naturally. It has been demonstrated to prevent the development of inflammation and cancer. Against bacteria, yeast, and fungi, anethole displays strong antibacterial properties. Both bacteriostatic and bactericidal activity against Salmonella enterica22 have been reported, although the use of fumigation to kill Salmonella has been shown to be ineffective23. Essential oil of P. anisum was found to have possible inhibitory effects on P. aeruginosa and B. subtilis, according to the current investigation. Essential oil from P. anisum had a maximum zone of inhibition against P. aeruginosa and B. subtilis of 19 mm in an agar disk diffusion test, which was equivalent to that of kanamycin (22 mm) and cephalexin (16 mm). These data also showed that essential oil from P. anisum, at doses of 0.003 and 0.007 g/ml, inhibited the development of P. aeruginosa and B. subtilis, respectively. In the study, the levels of MBC were equal in both of bacteria. The results demonstrate that the medicinal herb has antibacterial actions against both Gram-negative and Gram-positive pathogens, which is the whole point of the study.

The antibacterial properties of P. anisum have been noted by a number of authors. Using the disk diffusion method, the antibacterial effects of P. anisum fruit extracts in a variety of solvents were evaluated against four pathogenic bacteria (Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, and Klebsiella pneumoniae). Only aqueous and methanol extracts were found to exhibit moderate antibacterial activity against all of the test bacteria, with the former being more effective than the latter. On the other hand, acetone and petroleum ether extracts had no effect on the growth of the pathogenic test bacteria24. Antimicrobial activity of both water and ethanol extracts of P. anisum fructus was tested against Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis, Citrobacter koseri, Staphylococcus aureus, Streprococcus Pneumoniae, Enterobacter aerogenes, Micrococcus luteus, Staphylococcus Epidermidis and Candida albicans. Although the aqueous P. anisum fructus extract showed antimicrobial action against most bacteria, it was ineffective against Pseudomonas aeruginosa and Escherichia coli. As far as the antibacterial activity of isolated compounds, estragole inhibited all the tested.

Table 1: The diameters of growth inhibition zones in agar disk diffusion test in different dilutions of *P. anisum* essential oil.

Dilution(g/ml)	**Inhibition zone in disk diffusion (mm)**	
Microorganism	*P. aeruginosa*	***B. subtilis***
Positive control	22	**22**
1/32 (0.031)	19	**19**
1/64 (0.015)	19	**17**
1/128 (0.007)	12	**12**
1/256 (0.003)	9	**8**
1/512 (0.002)	8	**0**
1/1024 (0.001)	0	**0**
Negative control	0	**0**

Table 2: The diameters of growth inhibition zones in agar well diffusion test in different dilutions of *P. anisum* essential oil.

Dilution(g/ml)	**Inhibition zone in well diffusion (mm)**	
Microorganism	*P. aeruginosa*	***B. subtilis***
1/32 (0.031)	16	**16**
1/64 (0.015)	11	**11**
1/128 (0.007)	9	**8**
1/256 (0.003)	8	**8**
1/512 (0.002)	8	**0**
1/1024 (0.001)	0	**0**
Negative control	0	**0**

Table 3: MIC and MBC of essential oil of *P. anisum*.

Microorganism	*P. aeruginosa*	***B. subtilis***
MIC	1/256(0.003)	**1/128**

		(0.007)
MBC	**1/64 (0.015)**	**1/64** **(0.015)**

Strains; limonene showed an inhibitory action only on C. jejuni and L. monocytogenes and trans-anethole only inhibited C. jejuni26. Testing was done on Campylobacter jejuni, Listeria monocytogenes, and Salmonella enterica using a variety of plant essential oils and their separated constituents, including the oil from the P. anisum fruit. The antibacterial properties of P. anisum oil were demonstrated across the board. Essential oils and methanol extracts from various plants (including P. anisum) were tested for their bactericidal effects. Antibacterial activity was seen in both the essential oil and the methanol extract of these plants against the majority of the pathogens tested, with the greatest effect seen against Bacillus cereus and Proteus vulgaris. However, an additive impact against most tested bacteria27 was shown when essential oil and methanol extracts of these plants were combined. Last but not least, our findings corroborate those of previous researchers who discovered antibacterial activity in the essential oil of P. anisum against a wide variety of microorganisms, notably those resistant to numerous antibiotics. The antibacterial properties of P. anisum have been demonstrated against P. aeruginosa (PTCC No. 1707) and B. subtilis (ATCC No. 21332). The growth of both P. aeruginosa and B. subtilis were reduced by the essential oil tested, the results show that the essential oil has its own chemical composition, which may be connected with its antibacterial activity. The plant's essential oil had antibacterial characteristics, and these effects could be traced back to variations in the amounts and types of chemicals found in each variety's oil. It has potential as an antibacterial supplement in low-resource regions, paving the way for novel therapeutic agents to be developed [24-27].

References

1. Punasiya R, Joshi A, Sainkediya K, Tirole S, Joshi P, Das A, Yadav R. Evaluation of Antibacterial Activity of Various Extract of Hibiscus syriacus. Research Journal of Pharmacy and Technology. 2011; 4(5): 819-822.
2. Lakshmi Naidu PV, Kishore Kumar K, Mohan Kumar C, Gunesh G, Narasimha Rao M. Antimicrobial activity of Achyranthes aspera. Biosciences, Biotechnol Res Asia, 2006; 3(1a): 171-174.
3. Sofowora A. Medicinal plants and traditional medicine in Africa. Wiley, Nigeria, 1982: 33–34.
4. Rangarajan N, Sathiyamoorthy M. Phytochemical Screening and Antioxidant Studies in the Pulp Extracts of Cucurbita maxima. South Asian Journal of Engineering and Technology. 2016; 2(24): 131-140.
5. Nascimento G, Locatelli P, Freitas C Et al. Antibacterial Activity of Plant Extracts and Phytochemicals on Antibiotic Resistant Bacteria. Brazilian J. of Microbiology 2000; 31: 247-256.

6. Madhuri S, Pandey G. Some anticancer medicinal plants of foreign origin. Current Science, 2009; 96(6): 25.

7. Shakeri A, Hazeri N, Vlizadeh J, et al. Photochemical Screening, Antimicrobial and Antioxidant Activity ofAnabasis aphylla L. Extracts. Kragujevac J. of Science 2012; 34: 71-78.

8. Kalemba D, Kunicka. A. Antibacterial and antifungal properties of essential oils. Curr. Med.Chem. 2003; 10: 813-829.

9. Burt SA, Vlielander R, Haagsman HP, et al. Increase in activity of essential oil components carvacrol and thymol against Escherichia coli O157:H7 by addition of food stabilizers. J. Food Protect. 2005; 68: 919- 926.

10. Tunc I, Berger BM, Erler F, et al. Ovicidal activity of essential oils from five plants against two stored- products insects, J. of Stored Products Research, 2000; 36: 161-168.

11. Clinical and laboratory standards institute (CLSI). 2006; 26(2).

12. Shah B, Sheth F, Parabia M. Folk herbal knowledge on the management of respiratory disorders prevailing in ethnic society of Valsad district, Gujarat. Indian Journal of Natural Products and Resources, 2012; 3: 438-447.

13. Nenaah G. Antimicrobial activity of Calotropis procera Ait. (Asclepiadaceae) and isolation of four flavonoid glycosides as the active constituents. World Journal of Microbiology and Biotechnology, 2013; 29: 1255-1262.

14. Jones FA. Herbs – useful plants. Their role in history and today. Euro J Gastroenterol Hepatol, 1996; 8: 1227-1231.

15. Reynolds JEF. Martindale – the Extra Pharmacopoeia. 31st edition. London. Royal Pharmaceutical Society of Great Britain; 1996.

16. Lis-Balchin M, Deans SG. Bioactivity of selected plant essential oils against Listeria monocytogenes. J Appl Bacteriol, 1997; 82: 759-762.

17. Mitscher LA, Drake S, Gollapudi SR, Okwute SK. A modern look at folkloric use of anti-infective agents. J Nat Prod, 1987; 50: 1025-1040.

18. Zaika LL. Spices and herbs: their antibacterial activity and its determination. J Food Saf, 1988; 23: 97-118.

19. Omidbaigi R, Hadjiakhoondi A, Saharkhiz M. Changes in content and chemical composition of (Pimpinella anisum) oil at various harvest time. J. Essent. oil Bear Pl. 2003; 6: 46-50.

20. De M, De AK, Sen P, Banerjee AB. Antimicrobial properties of star anise (Illicium verum Hook f). Phytother Res. 2002; 16 (1): 94–5.

21. Kubo I, Fujita K. Naturally occurring anti-Salmonella agents. J. Agric. Food Chem. 2001; 49 (12): 5750–4.

22. Weissinger WR, McWatters KH, Beuchat LR. Evaluation of volatile chemical treatments for lethality to Salmonella on alfalfa seeds and sprouts. J. Food Prot. 2001; 64 (4): 442–50.

23. Fujita K, Fujita T, Kubo I. Anethole, a potential antimicrobial synergist, converts a fungistatic dodecanol to a fungicidal agent. Phytother Res. 2007; 21 (1): 47–51.

24. Akhtar A, Deshmukh A, Bhonsle AV, et al. “In vitro Antibacterial activity of Pimpinella anisum fruit extracts against some pathogenic bacteria,” VeterinaryWorld, 2008; 1(9): 272–274.
25. Gulcin I, Oktay M, Kirecci E, et al. Screening of antioxidant and antimicrobial activities of anise (Pimpinella anisum L.) seed extracts. Food Chemistry. 2002; 83: 371-382.
26. Friedman M, Henika PR, Mandrell RR. Bactericidal activities of plant essential oils and some of their isolated constituents against Campylobacter jejuni, Escherichia coli, Listeria monocytogenes and Fujimatu E, Ishikawa T, Kitajima J. Aromatic glucosides, alkyl glucoside and glucide from the fruit of anise. Phytochemistry. 2003; 63: 609-616.
27. Al-Bayati FA. “Synergistic antibacterial activity between Thymus vulgaris and Pimpinella anisum essential oils and methanol extracts,” J. of Ethnopharmacology, 2008; 116(3): 403–406.