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Running Head: Antimicrobial activity of Parsley Antimicrobial Activity of Essential oil of Parsley (PetroselinumCrispum) Against FoodPathogenic Bacteria

[1]Fereshteh Karimi, [1]Mohammad Rezaei, [1]Nabi Shariatifar, [1]Mehran Sayadi, [1]Issa Mohammadpourfard, [2]Ebadallahshiri Malekabad and [3]Hassan Jafari

[1]Department of Food Safety and Hygiene, school of public health,
Tehran University of Medical Sciences, Tehran, Iran
[2]Department of Epidemiology and Biostatistics, school of public health,
Tehran University of Medical Sciences (TUMS), Tehran, Iran
[3]General Physician, Arak University of Medical Sciences, Arak, Iran

Abstract: An experiment was conducted to find the antimicrobial activityof the essential oil from leaves and seeds of Parsley. The antimicrobial activity was examined using paper disc diffusion method and bymicro dilution technique against five pathogenic bacteria (Escherichia coli, Salmonella, Staphylococcus aureus, Yersinia and Vibrio cholera).The MICs (minimum inhibitory concentrations) of the PetroselinumCrispumseeds and leaves essential oil were 8, 0.25% against S. aureus, 4, 0.125% against V.cholera, 16, 0.5% against Yersinia enterocolitica and 32, 1% against the Salmonella enterica and E. coli, respectively.The results support the high efficacy of essential oils to control pathogenic bacteria and their use in developing new systems to prevent bacterial growth, extend the shelf life and increase the safety of the processed food.

Key words: Antimicrobial activity · Essential oils · Parsley · Seed · Leaf

INTRODUCTION

Food poisoning is yet a worry for people and the food producer notwithstanding the use of diverse preservation ways.Food safety researchers and regulatory organization are ceaselessly concerned with the extend and growing number of illness prevalence'scaused by some pathogenic and spoilage microorganisms in foods [1]. The increase of infections as a result antibiotic insistence microorganisms has entailed using new and natural antimicrobial substances [2]. One of the methods to keep safe food is used synthetic additives which reduce microbialgrowth or debar microorganisms and impeded or delay.Additives are harmful forhuman health particularly monosodium glutamate, aspartame, saccharin, sodiumcyclamate,sulfites,nitrates, nitrites and antibiotics. It bringsabout headache, nausea, weakness, mentalretardation, seizures, cancer and anorexia [3]. Because of the concern about the side effects of conventional preservatives and high attention of people to food safety,people have a better request to the use of natural products as areplacement to customary preservatives in the last few decades.

As a result of these, consumer's interest in natural products, particularly plant extracts, inclusive their essential oils and essences. Spices and herbs have been added to food for a wide variety of purposes for many thousands of years, for example to enhancethe flavor, color and aroma of food. As well as they are also known for their preservative and medicinal value[4]. The antimicrobial activities of plant may be due to a variety of different ingredients, including peptides, unsaturated long chain aldehydes, alkaloid components, some essential oils, phenols and water, ethanol, chloroform, methanol and butanol soluble constituents. These plants then appeared ascompounds with potentially considerable therapeutic application against humanpathogens, including bacteria, fungi or virus [5, 6] For example the crude methanolicextracts of neem plant have been shown to have strong antibacterial activity [7].

Corresponding Author: Nabi shariatifar, Department of Food Safety and Hygiene, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran. Tel: +989125091928.

In this paper the essentials oils from seed and leaf of parsley, were screened for their antibacterial activity against five pathogenic bacteria strains.

MATERIALS AND METHODS

Plant Samples: The different parts of parsley such as Seeds, leaves and stems were purchased from the market.Leavesand stems were washed with distilled water, dried in darken for at least 7 daysand then all parts of theplant were crushed to fine powder and stored at room temperature in the dark until used.Seeds were ground in a breaker and then keeping in laboratory until extraction procedure.

Extraction of Essential Oil: The seeds and leaves comminuted separately were placed in a hydro-distillation apparatus. For this purpose using a Clevenger apparatus for a minimum of 3h.Theessential oils poured in clean tubes, after the oils were dried over anhydrous sodium sulphate,they werestored in refrigerator until further analysis.

Microorganisms: Standard strains of microorganisms used in the present studyincluded Staphylococcus aureus ATCC 25913, Escherichia coli ATCC 8739, Salmonella enterica PTCC 1709, vibrio cholera PTCC 1611 and Yersinia enterocolitica PTCC 1477wasprepared Scientific and industrialresearch centerofIran.

Antimicrobial Activity Test

Assayby Disc Diffusion Method: The antibacterial activity of the essential oil was appraised by the paper-disk agar diffusion method against the microorganisms. Foreachtestantibacterialeffects,new cultures from 24 h were prepared.And Bacterialsuspensionswere dilutedwithnormalsaline,to obtain uniform suspensions of the Bacterial. tubes were incubated for 30 minat 37 °c. Final cell concentrations were about 108CFU/ml with reference tothe 0.5McFarland turbidomitry. The 0.1 ml of inoculum from the prepared culture was conveyed to Mueller-Hinton agar (MHA)medium. The inoculum was spread to surface of plates with a sterile swab.Sterilized filter paper disks (Whatman, 6 mm in diameter)were placed on the surface of the MHB and then by solvent dimethyl sulphoxide(DMSO)was prepared concentration of 10%,30% and 50% from the essential oil.The sterile discs were impregnated with 10μl volumes ofthe each concentration.

These plates were incubatedaerobicallyet 37^{0C} for 24 h.After incubationdiameters of bacterial growth inhibition zones around the paper disks were measured,recorded and data were analyzed.

Determination of Minimum Inhibitory Concentration (MIC): For quantitative tests to determine MIC, serial dilutionsfrom essential oil were made withMueller-Hinton Broth in a concentration rangefrom0.5 to 64%. The 96-well plates were preparedby dispensing into each well 100 µl of Mueller Hinton broth (MHB), 100 µl of the essential oiland 10µl of the inoculum.A positive control (containing100µlinoculum and 100 µl MHB) was included on each micro-plate. And then themicro-plateswere incubated at 37 °C for 24 h.The MIC was defined as the lowest concentrationof the compounds to inhibit the growth of microorganisms.The experiment was carried out intriplicate.

Minimum Bactericidal Concentration (MBC) of Essential Oil: MBC was determined by sub-culturing the 5 µlof test dilution from each well (in the leastDilution noturbidity,was observed) on to aMueller HintonAgar (MHA) platesand incubating at 37 °C for 24h. The complete absence of growth at applied concentrationwas considered as the minimum bactericidal concentration.

RESULTS ANDDISCUSSION

Determination of MIC and MBC values: MIC of the essential oilfrom seeds of parsley varied from 4to 32%.And MIC of oil from leaves of parsley for pathogenic bacteria ranged from 0.5to1%.MBC value of parsley essential oil from seeds was obtained lowestagainst vibriocholera(8%), while it was obtained 16%against Staphylococcus aureusand has shown intermediate effect against Yersinia, Whilehighest (64%) in cases of Escherichia coli and Salmonella. MBC value of the oil fromleaves,showed inhibitory effect in concentration from 0.125 to 1%(Table 1).

Inhibition Zone Diameter: The antibacterial activity of the essential oil from seeds and leavesof parsley was recognized by the presence or absence of inhibition zone and measuring the diameter of the inhibition zone around discs.essential oil from seed of parsley has shown 11mm inhibitionzone diameter against Staphylococcus aureus,10 mm against Escherichiacoli and Yersinia, 10.5mm against vibriocholera, While low growth inhibition zone diameters

Table 1: Determination of MIC and MBC value (%) for parsley leaves and seedsessential oil against pathogenic bacterial strains

	staphylococcus aureus		vibrio cholera		Yersinia		Escherichia coli		Salmonella	
Test	Seed	Leaf	Seed	Leaf	Seed	Leaf	Seed	Leaf	Seed	Leaf
MIC (%)	8	0.25	4	0.125	16	0.5	32	1	32	1
MBC (%)	16	0.125	8	0.125	32	0.25	64	1	64	0.5

Table 2: comparison of average inhibitory halo diameter (mm) of various bacterial strains for parsley seed essential oil

Bacterial strain	Samples	Min	Max	Average ± SD
Staphylococcus aureus	3	10	11.5	11 ± 0.87
Escherichia coli	3	9.5	11	10 ± 0.87
Vibrio cholera	3	9.5	11.5	10.33 ± 1.04
Yersinia	3	9	11.5	10 ± 1.32
Salmonella	3	8.5	10	9 ± 0.87

Table 3: comparison of average inhibitory halo diameter (mm) of various bacterial strains for parsley leaf essential oil

Bacterial strain	Samples	Min	Max	Average ± SD
Staphylococcus aureus	3	13.5	15	14.5 ± 0.87
Escherichia coli	3	13	14	13.5 ± 0.5
Vibrio cholera	3	12.5	15	14 ± 1.32
Yersinia	3	13	15.5	14.5 ± 1.32
Salmonella	3	12.5	12.5	12 ± 0.5

against Salmonella (9mm) (Table 2) and inhibition zone diameter of the essential oil from leaves varied from 12 to 14.5mm (Table 3).

Equal to increment the resistance of microorganisms to antibiotics currently used, Side effects and high cost of synthetic compounds, as a result of this, researchers are looking for natural products.

The results of this study showed that essential oil from the parsleyinhibited the growthof five bacteriaEscherichia coli,Salmonella, Staphylococcusaureus, Yersinia enterocolitica and vibrioparahaemolyticus. It is proved by differentMIC and MBC values obtained in essential oil when used against each bacterial culture.The MIC value of the essential oil from seed and leaf of parsley wereobtained lowest against V.cholera,while highest The MIC value in case of E.coliand Salmonella.Andessential oil tested has shownhigher MBC values than MIC values against eachbacterial strain.MBCvalue of the essential oil from seed of parsley wasfound to be lowest (8%) against vibriocholera and highest MBC value against E.coliandSalmonella.While lowest and highest MBC value from leaf of parsley was obtained againstS.aureus, V.cholera(0.125%) and E.coli(1%).

Further effectiveness of essential oil was determined by agar disc diffusion method and inhibition zone diameters were measured in presence and absence of essential oil. Highest inhibitionzone diameters of the essential oils from seed and leaf ofparsley were obtained byS.aureusand lowestinhibitionzone diameters were obtained by Salmonella.

In another work[8] has been reported that ethanolic extract of parsley seed had inhibitory effect at various concentration(0.1 to 0.4 gr.ml^{-1}) againstGram negative Br.melitensis,E.coli,P.mirabilis,P.aeruginasa) andGram positive.It was effective in high concentration (0.4gr.ml^{-1}) on Salmonella typhi.Ethanolicextract of parsley didn't inhibit the growth of B.subtilis,B.bronshiseptica and S.ureus. Similarly [9]showedthat aqueous extract of the parsley leaf no inhibitory effect at various concentrationon(E.coli,Salmonellatyphi,S.aureusand other bacteria studied).

However, only the concentration40mg/ml was effective on P. aeruginosaButphenolic extract of the parsley in concentration (>=20 mg.ml^{-1}) had inhibitory effect againstE.coli, Salmonella typhi.Proteus mirabilis, Pseudomonas eruginosa.While no inhibitory effect on S.aureus.Andmethanolic extract of parsley has inhibitory effect against *P.* aeruginosaand Staph. ureus, Enterococcus and Salmonella typhi.And also ethanolic extract of parsley no inhibitory effect on S.aureus, On the other hand E.coli,Salmonella typhiand Pseudomonas

aeruginosawere affected by the ethanolic extract of parsley at >=10 mg.ml^{-1}More0verethanolic extract of parsley at the concentrationof 500 mg•ml-1 exhibited antibacterial effect against Proteus Mirabilis and in the concentration (>=100 mg.ml^{-1}) had inhibitory effect on Staphylococcusaureus, while E.coli,B. Cereus, E. Faecalis, S. Choleraesuiswereresistantto all concentrations used [10]. Similar antimicrobial activity is reportedin essential oil act of the parsley at a dose of 40 μl had inhibitory effect on listeria innocua and it was not active in the inhibition of S. marcescens and P. fluorescens at all added doses[11]According to a report of [12]. Methanolic extract of parsley (petroselinumcripsum) leafe had inhibitory effect against Bacillussubtilis, Staphylococcus aureus, Micrococcus luteus, Escherichia coli, Psuedomonosaeruginosa and Sallmonellatyphi. The comparison ofour resultswiththesestudies showed that the essential oils from Seed and leaf of parsley have stronger anti bacterial effect than the extract.

CONCLUSION

In the present study, essential oil tested has shown a variable degree of antimicrobialactivity on different microorganisms. Therefore using essential oil of parsley as antimicrobial additives in food may be useful and alternative medical therapy formicroorganisms which may resist customary treatment.This willsuggestion a great helpin facingthe appearance spread of bacteria.

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