Journal of Medical Sciences

ISSN 1682-4474

science alert

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Research Paper

JMS (ISSN 1682-4474) is an International, peer-reviewed scientific journal that publishes original article in experimental & clinical medicine and related disciplines such as molecular biology, biochemistry, genetics, biophysics, bio-and medical technology. JMS is issued four times per year on paper and in electronic format.

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Shahla Mansouri
Department of Microbiology
Kerman University of Medical Sciences
P.O. Box 444
Kerman, Iran

Tel: +98 341 3221665
Fax: +98 341 3221671

J. Med. Sci., 5 (3): 216-221
July-September, 2005

Inhibitory Effect of Some Medicinal Plants from Iran on Swarming Motility of *Proteus* Rods

Shahla Mansouri, Afshin Amari and Ali Ghanberi Asad

In order to develop a media to abolish the swarming of *proteus* without effecting the growth of other bacteria, the anti-swarming activity of 25 plant essential oils or extracts were evaluated on standard and clinical isolates of *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis, P. vulgaris* and *P. Penneri*, using standard agar dilution method. The most effective anti-swarming agents tested were; the mixture containing equal amounts of essential oil of *Ferula gumosa Boiss* plus *Lavandula officinalis* and essential oil of *Zataria multiflora Boiss.* Essential oil of *Zataria multiflora Boiss* had antibacterial activity and inhibited the growth of many isolates at concentration of 2%. Whereas the essential oil of Lavandula *officinalis* plus *Ferula gumosa Boiss* although had no antibacterial activity at this concentration, completely inhibited the swarming behavior of all the *proteus* strains tested. This mixture was quite active in the presence of 7.5% blood as in blood agar plates. Essential oil of *Zataria multiflora Boiss* and *Lavandula officinalis* plus *Ferula gumosa Boiss* with good anti-swarming activities and long shelf life, are commercially available and can be used as an effective anti-swarming agent for preparation of media in clinical laboratories for the isolation of more fastidious bacteria in a mixed culture with *proteus.*

Key words: Swarming, *proteus,* plant extract, essential oil

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Department of Microbiology, Kerman University of Medical Sciences,
P.O. Box 444, Kerman, Iran

INTRODUCTION

The genus *Proteus* of the *Enterobacteriaceae* family consists of four species, *P. mirabilis, P. vulgaris, P. penneri, P. hauser* and three unnamed genomospecies[1]. These bacteria are widely distributed in natural environments, such as polluted water, soil and manure, where they play an important role in decomposing organic matter of animal origin[2]. Besides the saprophytic mode of life in the natural environments and in the intestine of humans, wild and domestic animals, they are well known opportunistic pathogens and the first three species are involved in urinary tract infections[3]. The most characteristic feature of this genus is the swarming motility[4,5]. This phenomenon or zonal growth is described as the formation of concentric zones of bacterial growth, able to cover the whole surface of solid culture medium[5]. Swarming frequently interferes with the enumeration of other microorganisms in clinical microbiology investigation and enhances the difficulty of isolating organisms from mixed cultures[6]. For example in veterinary microbiology, samples taken from ears of cats and dogs are often contaminated with *P. mirabilis* and the swarming motility makes it almost difficult or even impossible to isolate single colonies of other bacterial species present in the sample[7]. Many methods and addition of many chemical substances to the agar medium are recommended in order to inhibit the swarming. For example, the use of very dry medium, increasing the concentration of agar, addition of urea, p-nitrophenylglycerol, ethanol, activated charcoal, sodium azide, certain amino acids and ferrous ions[3,5-8]. However most of the works were performed only on reference bacterial strains and the effect of these substances on other pathogens remain largely unknown[5]. Here we describe the inhibitive effect of 25 essential oils or plant extracts on swarming behavior of five strains of *P. mirabilis*, two strains of *P. vulgaris,* two strains of *P. penneri* and the growth of *E. coli, P. aeruginosa* and *S. aureus* as the most frequently isolated bacteria in the diagnostic laboratories.

MATERIALS AND METHODS

Bacterial strains: A standard strain of *P. mirabilis* (ATCC 43071) was obtained from Mast Diagnostics (France), other strains including *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeruginosa* (ATCC 27853) were obtained from Persian Type Culture Collections, Tehran, Iran. Two strains of *P. penneri,* two strains of *P. vulgaris* and four strains of *P. mirabilis* were isolated from urinary tract infections in Kerman, Iran and were identified by standard biochemical methods[9].

Plant extract and essential oils: Methanolic extracts of *Zataria multiflora, Eucalyptus globolus* (Labill), *Echium ammonium* Fisch and Mey and *Glycyrrhizia glabra* L., were prepared as mentioned earlier[10] other plant extracts and essential oils were obtained in a liquid form from Barij Essence Kashan, Iran (Table 1).

Assay for swarming inhibition: Standard NCCLS agar dilution method was used to test the anti-swarming activity of the plant extracts or essential oils[11]. Stock solutions of dried extracts were made in DMSO and the extracts (0.1 to 0.4 mg mL^{-1} final concentrations) were added to the Muller Hinton agar (Merck, Germany) after sterilization, at 60°C. Other plant extracts or essential oils (commercial types) were added by the same method to the medium at final concentrations of 0.5 to 2% v/v. Plates were inoculated with 10 μL (10^5 CFU mL^{-1}) of each organisms, incubated at 37°C and the growth and swarming was monitored 24 h later. Muller Hinton agar without the plant extracts was used as the control of growth and DMSO at its highest concentrations used was tested to see if it had any effect on the growth or the swarming.

RESULTS

From 25 essential oil or methanolic plant extracts used, 13 essential oils or extracts had no affect either on the swarming or growth of bacterial strains tested (Table 1). Other essential oils or extracts either effected the growth or the swarming behavior of some bacterial strains or changed the pattern of swarming. *Myrtus communis* L., essential oil (1.5% v/v) or extract (2 mg mL^{-1}) inhibited the growth of *S. aureus,* 3 strains of *P. mirabilis* including the standard strain and one strain of *P. vulgaris* but had no effect on the swarming or growth of other tested bacteria. Essential oil of *Gumium cyminum* L. (2% v/v), slightly inhibited the growth of *S. aureus,* but did not change the swarming (Fig. 1A). In the presence of essential oil of *Rosa damascene* L., (2% v/v) *P. vulgaris* isolates did not swarm (Fig. 1B). Essential oil of *Anethum graveolens* L., inhibited the swarming of *P. vulgaris* and *P. penneri* strains plus two strains of *P. mirabilis*, other strains swarmed on the medium, forming a turbid membrane on the agar surface which masked the original spot of

Table 1: Evaluation of the effect of native medicinal plants of Iran used as essential oils (1.25-1.5% W/W) or extracts (0.1- 0.4 mg mL^{-1}) on swarming or growth of 9 strains of proteus plus standard strains of *E. coli*, *S. aureus* and *P. aeruginosa*

Plant family	Plant species	English common name	Trade name	Type	Inhibition of swarming	Inhibition of growth
Apiaceae/Umbelliferae	*Anethum graveolens* L.	Dill, Dill Weed, Dill herb	Dillsun oral drop	Essential oil	Yes	No
	Ferula gumosa Boiss	Galbanium anti acne	Lotion Essential oil	Essential oil	Yes +	No
	Foeniculum vulgare	Sweet fennel	Oral drop fennilin	Essential oil	No	No
	Gumium cymium L.	Gumium plant	Oral drop	Essential oil	Yes +¤	No
	Coriander sativum	Coriander	Drop coriander extract	Extract	No	No
Boraginaceae	*Echium amoenum* Fisch and Mey	Alkanna linctoria	-	Extract	No	No
Compositae	*Artemisia draconculus*	Tarragon	Tarragon C.W.2%	Extract	No	No
	Cynara Scolymus	Artichoke	Oral drop cynabile	Extract 1:1	No	No
	Artemisia sieberi	Worm wood	Inhaler Artemisia C.W	Pure essential oil	Yes+	No
	Matricaria chamomilla	Chamomilla	Matrica mouth wash	Liquid extract 1:2	No	No
Geraniaceae	*Pelagonium roseum*	Geranium	Lotion Geranium	Essential oil 2%	No	No
Labiatae	*Lavandula officinalis*	Lavender	Lotion Essential oil	Essential oil	Yes+	No
	Mentha spicata	Spearmint	Oral drop supermint	Essential oil	No	No
	Mentha polegium	Pudding grass	Drop pennyroyal C.W	Essential oil	No	No
	Teucrium polium	Germander (golden wooly plant)	Oral drop teucrium	Extract	No	No
	Zataria multiflora Boiss	Avisham	Oral drop gasterolit	Essential oil	Yes+	Yes
	Zataria multiflora Boiss	Avisham	-	Extract	No	Yes
Leguminosae	*Glycyrrhizia glabra* L.	Licoric	-	Extract	Yes+	No
	Lawsonia inermis L.	Henna plant	Lotion Henna	extract	Yes+	Yes
	Lawsonia inermis L.	Henna plant	Lotion	Liqiud extract	Yes+	Yes
Myrtaceae	*Myrtus communis* L.	Myrtle	Essential oil of myrtle	Essential oil	No	No
	Myrtus communis L.	Myrtle	-	Extract	No	Yes
	Eucalyptus globolus (Labill)	Eucalyptus	-	Extract	No	Yes
Rosaceae	*Rosa damascene*.L.	Damaskrose	C.W Rose oral drop	Essential oil	Yes	No
Rutaceae	*Citrus aurantifolia*	Lime	Oral drop lime	Essential oil	No	No
	Citrus aurantium	Orange	Orange oil	Essential oil	No	No

Yes: The swarming pattern was changed; Yes$^+$: The pattern was changed and swarming of some strains was inhibited; No: No difference with the control

bacterial inoculation. *Artemisia sieberi* at concentration of 2% (v/v) also inhibited the swarming of both *P. vulgaris* strains, one strain of *P. penneri* and two strains of *P. mirabilis*. Extract of *Eucalyptus globolous* (Labill), slightly inhibited the growth of *S. aureus* and *E. coli* but had no effect on the growth or swarming of other strains. *Lawsonia inermis* L., at the concentration of 1% (v/v) had inhibitory effect on *S. aureus*, one strain of *P. vulgaris* and one strain of *P. penneri*. The other strains of *P. vulgaris* and *P. penneri* had very limited growth (Fig. 1C). In this medium, one strain of *P. mirabilis* had the typical swarming which covered nearly the entire plate. At a concentration of 1.25% (v/v) of this extract only four bacterial strains had obvious growth and one strain of *P. penneri* swarmed, although the colony size was small (Fig. 1D). In a medium containing 2% (v/v) liquid extract of *Lawsonia inermis* L., the growth of all bacteria except *E. coli* was inhibited. In the presence of the essential oil of *Glycerrihizia glabra*, (concentrations of 0.5 to 2% v/v) all the bacteria were able to grow and swarming for all the strains were in a form of thick homogenous zone of bacterial growth (Fig. 1E).

Methanolic extract of *Zataria multiflora* at different concentrations had no effect either on growth or swarming of the isolates, whereas the essential oil of *Zataria multiflora* was among the most active plant materials tested. At the concentration of 1.25% (v/v), all the bacteria were able to grow but only two strains of *P. mirabilis* and one strain of *P. penneri* swarmed (Fig. 1F). When the concentration of this extract was raised to 2% (v/v), the swarming for all of the strains were completely inhibited. However at this concentration one strain of *P. penneri*, the standard strain of *P. mirabilis* and *S. aureus* failed to grow. *Zataria multiflora* at higher concentrations (2%) had growth inhibitory effects for many bacterial strains tested. This essential oil (concentration of 1.25 and 1.5% v/v) had slight effect on the swarming when the medium was supplemented with 5-7.5% blood.

In presence of a mixture containing equal amount of essential oil of *Lavandula officinalis*, plus *Ferula gumosa* boiss, (Galbanium anti acne), although the bacterial growth was not inhibited, swarming was abolished and only one strain of *P. penneri* and one strain of *P. mirabilis* showed a very slight swarming. At a concentration of 2% (v/v) all the isolates were able to grow and there was no swarming. This medium preserved its anti-swarming activity when the medium was supplemented with blood and the hemolysin production on this medium was very clear such as the original blood agar plates.

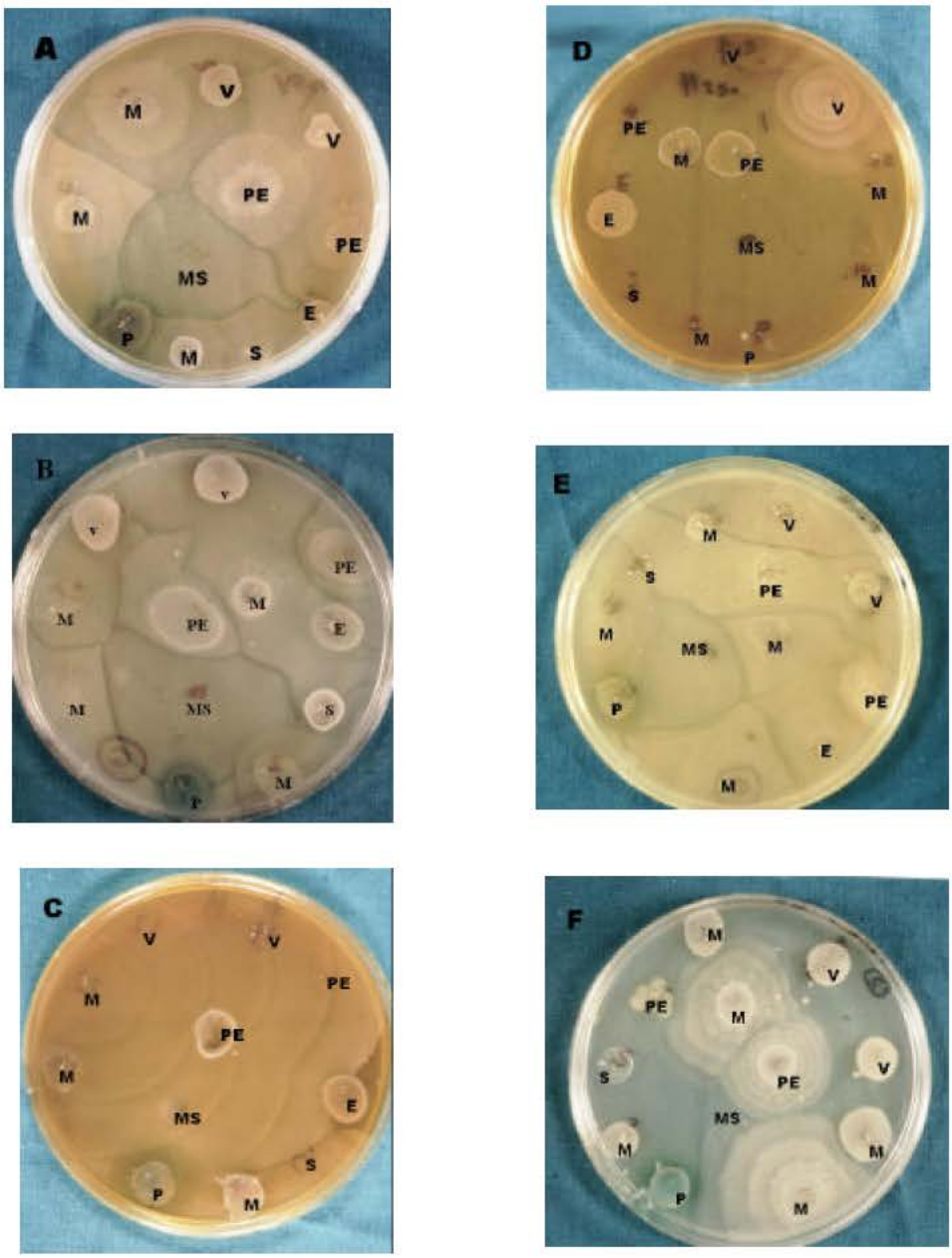

Fig. 1: Effect of several plant extracts and essential oils on growth of 12 different bacterial species and swarming behavior of *Proteus mirabilis, P. vulgaris* and *P. penneri*. A) Essential oil of *Gumium cymium* L., B) Essential oil of *Rosa damascene;* C) Fluid extract of *Lawsonia inermis* L., D) Fluid extract of *Lawsonia inermis* L., E) Methanolic extract of *Glycyrrhizia glabra* L. and F) Essential oil of *Zataria multiflora Boiss,* at concentration of (v/v) of 1.5, 1, 1.25, 2 and 1.25%, respectively. Plate abbreviations indicated are: M: *Protues mirabilis,* V: *Proteus vulgaris,* PE: *Proteus penneri,* E: *Escherichia coli,* ATCC 25922; S: *Staphylococcus aureus,* ATCC 25923: MS *Protues mirabilis,* ATCC 43071 and P: *Pseudomonas aeruginosa* ATCC 27853

DISCUSSION

Proteus rods had proteolytic and hemolytic activities, having considerable resistance to sodium chloride and are grouped among important putrefying bacteria[10,12]. *P. mirabilis* and *P. vulgaris* are also well known opportunistic pathogens, causing urinary tract infections and are also responsible for systemic and localized infections[3,6]. Several potential virulence factors may be responsible for the pathogenic properties of these bacteria[7,13]. It has been shown that swarming differentiation and expression of virulence factors, such as protease, hemolysin and urease, are co-ordinately regulated in *P. mirabilis*[5,8,13]. Recurrent urinary tract infections usually had a poly microbial origin and the identification of all the bacteria involved in the infection is important. Therefore swarming of *Proteus* in solid media used for the isolation of these bacteria may over whelm the growth of other pathogens thereby annulling plans for adequate antibiotic options[5].

Many reports show the effectiveness of traditional herbs against microorganisms[2,14,15]. Plants are indispensable source of chemical compounds and there are many interests to investigate natural material as a source of new antibacterial agents. The aim of this study was to find a natural non toxic compound that can promote the growth of pathogens but inhibit swarming.

It has been shown that swarming of different strains of *proteus* may fail to penetrate into each other and a line of demarcation is produced between the two swarmers (Dienes phenomenon)[3]. The interesting finding in this study was the effect of plant materials on the pattern of swarming and Dienes phenomenon. For example in the presence of essential oil of *Gumium cyminum* L., swarm cells of *P. mirabilis* and *P. penneri* spread outwards and formed the synchronous oscillating waves around the colonies (Fig. 1A). However in the medium containing the essential oil of *Rosa damascene* L., swarming pattern was changed and a homogenous expanding zone of growth was seen around the colonies (Fig. 1B). The place of demarcation line could be also compared in Fig. 1A and 1B. Since the production of this line is unrelated to the flagellar antigen of the strain, but appears to depend both on the bacteriocin produced and the bacteriocin to which the bacteria are sensitive[3], its possible that these plant materials, increased or decreased the production of bacteriocin in some strains.

The most active plant materials tested were the essential oil of *Zataria multiflora* and a mixture containing equal amount of essential oil of *Lavandula officinalis,* plus *Ferula gumosa Boiss*, (Galbanium anti acne). The essential oil of *Zataria multiflora* had both antibacterial and anti-swarming activity, but its anti-swarming activity could not be applied to blood agar plates. Therefore the mixture containing essential oil of *Lavandula officinalis* and *Ferula gumosa Boiss,* has superiority to the essential oil of *Zataria multiflora* in respect to swarming, since it has no antibacterial activity and the anti-swarming property of this mixture is quite active in presence of blood, it can be applied to blood agar plates in order to support the growth of fastidious bacteria.

Effect of these plant materials on swarming and growth inhibition is not clear and further works are required to find the active constituent of plant material to clarify their mechanism of action. However since the non-swarmers are not virulent, these essential oils especially of *Zataria multiflora* which is generally used as an oral medicinal plant, can be used for food spoilage control, cosmetics and pharmaceutical use. Further studies will be required to elucidate the cell target of the essential oil components.

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