

Antibacterial Activity of Some Folklore Medicinal Plants Used by Bakhtiari Tribal in Southwest Iran

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Abstract

In this study, antibacterial activity of ethanol extract and essential oil of 10 Iranian folklore herbs including, *Heracleum lasiopetalum* Boiss., *Satureja bachtiarica* Bunge., *Thymus daenensis* Celak., *Ziziphora tenuifolia* L., *Echiophora platyloba* L., *Dracocephalum multicaule* Benth., *Kelussia odoratissima* Mozff., *Mentha longifolia* Hudson., *Achillea kellalensis* Boiss. and *Stachys lavandulifolia* Vahl. were investigated against of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* by agar disc diffusion and serial dilution assays. Most of the extracts and essential oils showed relatively high antibacterial activity against all the tested bacteria with the diameter of inhibition zone ranging between 8 and 23 mm. Of the plants studied, the most active extracts were those obtained from essential oil of *Satureja bachtiarica* and *Thymus daenensis*. The MIC values for active extract and essential oil ranging between 0.039 and 10 mg/ml. The results obtained appeared to confirm the antibacterial potential of the plants investigated. In conclusion it can be said that the extract and essential oil of *Satureja bachtiarica* and *Thymus daenensis* could be used as natural antibacterial agents in the food preservation and human health.

Keywords: Medicinal plants, Iranian folklore herbs, Antibacterial activity

1. Introduction

Staphylococcus aureus, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella* (over 1600 types), *Streptococci* and etc. are common food-borne pathogenic bacteria and are frequently isolated from various foods, including meat, milk and milk products, seafood and vegetables. Food poisoning originating from contaminated foods by both Gram-positive and Gram-negative bacteria causes concern to society and to the industry.

Even today, plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries (Zakaria, 1991). Medicinal herbs contain physiologically active principles that over the

years have been exploited in traditional medicine for the treatment of various ailments as they contain anti-microbial properties (Kelmanson et al., 2000; Srinivasan et al., 2001). Antimicrobial properties of herbs have been documented in ancient literature and the interest continues to the present. However, few of these have been investigated for their antimicrobial.

Spices are herbal products which have been safely used by people around the world to impart desirable flavors and aromas to the local foods. It looks that there has been a natural selection for spices as these products are mainly originated from plants grown in the tropical regions with wide distribution of food-borne bacteria. Several of these spices and their essential oils have been reported to possess antimicrobial activities including garlic, savory, basil, laurel, mint, cumin, onion, sumac and thyme (Aktug and Karapinar, 1986; Arora and Kaur, 1999; Delgado et al., 2004; El-Khateib and Abd El-Rahman, 1987; Nasar-Abbas and Kadir Halkman, 2004; Ozcan and Erkmen, 2001; Shelef, 1983).

Numerous Iranian folklore herbs for example: *Heracleum lasiopetalum*, *Satureja bachtiarica*, *Thymus daenensis*, *Ziziphora tenuifolia*, *Echiophora platyloba*, *Dracocephalum multicaule*, *Kelussia odoretascima*, *Mentha longifolia*, *Achillea kellalensis* and *Stachys lavandulifolia* have been utilized as traditional medicines by the indigenous people of Chaharmahal va Bakhtiari, Iran (Ghasemi Pirbalouti, 2009). Hence, it is necessary to establish the scientific basis for the therapeutic actions of traditional plant medicines as these may serve as the source for the development of more effective drugs. The research reports on these folklore herbs were limited and the anti-bacterial effects of ten herbs have not been studied until now. In this study, it was aimed to determine antibacterial activity of ethanol extract and essential oils of 10 plant species which are Iranian endemic plants.

2. Experimental

2.1 Plant material

An ethnobotanical survey was conducted in Chaharmahal va Bakhtiari Province, South-West of Iran. The survey was conducted by interviewing traditional healers in each locality using the local language. Each interview followed a semi-structured questionnaire designed to obtain the following information: scientific and local plant names; habit; plant parts used; uses/ailments treated. The plants were collected from mountain areas of Zagross, Chaharmahal va Bakhtiari district, during May–Sep, 2008. Their identity was confirmed and voucher specimens were deposited at the Researches Centre of Medicinal and Aromatic Plants, Islamic Azad University, Shahrekord Branch, Iran.

2.2 Extract preparation

Dried plant material were powdered (200g) and subjected to hydro-distillation (2000 ml distilled water) for 4 h using a Clevenger-type apparatus according to the method recommended in British pharmacopoeia (British Pharmacopoeia, 1988). Leaves and flowers of some of the plants shade dried and ground into a powder (100g), macerated in 200 ml of ethanol 80% and filtered were dried at 35°C under rotary vacuum (Model Zirbus 302®, Italy). The extract samples were stored in universal bottles and refrigerated at 4°C prior to use.

2.3 Bacterial strain

One Gram-positive (*Staphylococcus aureus*) and three Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) bacterial strains were all clinical isolates obtained from Food Microbiology Laboratory, Veterinary Medicine Faculty, Islamic Azad University of Shahrekord Branch, Iran and identified using conventional morphological as well as biochemical tests. Stock cultures of bacteria were kept in 20% glycerol PBS (phosphate buffered saline) at -70 °C. Active cultures were generated by inoculating 100 µl of the thawed microbial stock suspensions into 5ml nutrient broth (Merck, Germany) followed by overnight incubation at 37 °C. An initial bacterial suspension containing 10⁷CFU/ml was made from the flask broth culture. Subsequent dilutions were made from the above suspension, which were then used in tests.

2.4 Antibacterial test

2.4.1 Disc diffusion assay

The disc diffusion method of Iennette (1985) was used with some modification to determinate rate of inhibition growth of bacteria by plant extract and essential oil. BHI agar (Merck, Germany) was used to prepare the culture medium and autoclaved at 121°C for 15 min. Briefly, plates (8-cm diameter) were prepared with 10 ml agar inoculated with 1 ml of each bacterial suspension. Sterile paper discs (6 mm in diameter) were impregnated with 60 µl of dilutions of known extract concentrations (100 µg/disc) and incubated at 35°C for 18 h. The extracts were dissolved in dimethyl sulfoxid (DMSO, 15 µl) before the test for antimicrobial activity. Discs (6 mm diameter) of ampicillin, erythromycin and ciprofloxacin (10 µg) were used as positive controls. Bacterial growth

inhibition was determined as the diameter of the inhibition zones around the discs (mm). The growth inhibition diameter was an average of three measurements, taken at three different directions. All tests were performed in triplicate.

2.4.2 Serial dilution

The minimal inhibitory concentration (MIC) and the minimal bactericidal concentration (MBC) values were determined by serial dilution assay. The MIC was defined as the lowest concentration of the compound to inhibit the growth 50% of microorganisms and the MBC was defined as the lowest concentration of the compound to kill the microorganisms. All extracts were initially tested at 10000 µg/ml and serially diluted to 39 µg/ml. Each tube was inoculated with 5 ml of bacterial suspension at a density of 10^7 CFU/ml and incubated at 37° C for 48 h. The growth of microorganisms was observed as turbidity determined by the measure optical density at 600 nm, by spectrophotometer (Eppendorf, AG, Germany). Erythromycin was included as positive control in each assay. Extract-free solution was used as a negative control. Control tubes were incubated under the same condition. All assays were carried out in triplicate. The inhibition demonstrated by the extracts is expressed by the following equation (Zampini et al., 2005):

$$\text{Inhibition \%} = [(OD_c - OD_t) / OD_c] \times 100$$

Where OD_c is the OD_{600} for the negative control (containing no extract) and OD_t is the OD_{600} for the sample treated with the antimicrobial compounds.

3. Results

3.1 Ethnobotanical survey

The results of the survey are presented in table 1.

3.2 Antibacterial test

3.2.1 Disc diffusion method

The Growth inhibition value of extract and essential oil on bacteria strains are shown in table 2. The extracts from different plant species studied showed antibacterial activities, with the diameters of inhibition zone ranging from 8 to 23 mm. There were significant differences ($P \leq 0.05$) in the antibacterial activities of plant extracts. Among the plants tested essential oil of *Satureja bachtiarica* and *Thymus daenensis* showed the best antibacterial activity. The result showed that most of the extract and essential oils could effectively inhibit the growth of *Escherichia coli*. Among these, the extracts of *Satureja bachtiarica*, *Dracocephalum multicaule* and *Achillea kellerensis* and essential oil of *Satureja bachtiarica* showed strong inhibitory (Table 2 & Fig 1).

Among the plants tested only essential oil of *Satureja bachtiarica* and *Thymus daenensis* could effectively inhibit the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Interestingly, essential oil of *Thymus daenensis* and *Kelussia odoretascima* showed promising antibacterial activities against *Klebsiella pneumoniae* (Table 2 & Fig 1).

3.2.2 Serial dilution

Subsequent experiments were conducted to determine minimal inhibitory concentration and minimal bactericidal concentration of all selected plant extracts and essential oils. The results are presented in Table 4. Among the plants tested, *T. daenensis* and *S. bachtiarica* showed the best antibacterial activities. Also, essential oils of *H. lasiopetalum*, *K. odoretascima* and *A. kellerensis* showed promising antibacterial activities against *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* (Table 4). The MIC (>50% growth inhibition) for extracts and essential oils presented in Table 4. The MIC values for active extract and essential oil ranging between 0.039 and 10 mg/ml. The results obtained appeared to confirm the antibacterial potential of the plants investigated. The essential oil *S. bachtiarica* showed the best MIC value and activity against four bacteria strains used.

4. Discussion

The results showed that Gram- negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) were more sensitive than Gram-positive bacteria (*Staphylococcus aureus*) (Table 2-4). Antibacterial activity of extract and essential oil of plants varied related to the test organisms. The most active of the concentrations was 10 mg/ml concentration inhibiting completely the growth of all the Gram- negative bacteria. Whiles, Cos et al., 2006 reported that Gram-negative bacteria are generally more resistant compared to the Gram-positive ones.

Pervious works (Ghasemi Pirbalouti et al., 2009) showed that essential oils of *Thymus daenensis* and *Thymus* spp. (Elam) flowers exhibited antibacterial activities against *Listeria monocytogenes* from chicken meat. In a previous study, the minimum inhibitory concentration (MIC > 50 % growth inhabitation) against *Listeria monocytogenes* for *Thymus daenensis* and *Thymus* spp. (Elam ecotype) were 700 and 1700 µg/ml, respectively.

Fazeli et al., 2007 studied antimicrobial effects of two medicinal plants (*Rhus coriaria* L. and *Zataria multiflora* Boiss.) used in Iranian traditional medicine were investigated against some pathogenic food-borne bacteria. The minimum inhibitory concentrations of *Rhus coriaria* and *Zataria multiflora* were determined against several strains of Gram-positive and Gram-negative bacteria. They have reported that *Bacillus cereus* was found to be the most sensitive bacteria to *Rhus coriaria* showing the MIC of 0.05%, while *Staphylococcus aureus* and *Proteus vulgaris* ranked next with 0.10% followed by *Shigella flexneri*, *Escherichia coli* and *Salmonella typhi* with MIC of 0.20%.

According to a report (Rasooli et al., 2006) extract and essential oils of *Thymus eriocalyx* and *Thymus porlock* inhibited the growth of *Listeria monocytogenes*. The essential oil and extract of some aromatic plants (for example mint family, *Lamiaceae*) with a higher percentage of cavracrol and thymol have a higher efficacy against strain bacterial (Rasooli et al., 2006).

5. Conclusion

According to the findings of this study, the essential oil *S. bachtiarica* and *T. daenensis* leaves and flowers had antibacterial activities. The present study suggests that the essential oil of these plants is a potential source of natural antibacterial agents. After this screening experiment, further work should be performed to describe the antibacterial activities in more detail as in vivo. Also phytochemical studies will be necessary to isolate the active constituents and evaluate the antibacterial activities against a wide rang of bacteria population.

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Table 1. Ethnobotany of Iranian medicinal plants used in this study

Scientific name	Family name	Local name	Habit*	Parts used	Uses/ailments treated
<i>Achillea kellalensis</i> Boiss. & Hausskn.	Asteraceae	Golberenjaz	H	flowers	Wound, carminative, indigestion
<i>Dracocephalum multicaule</i> Montbr & Auch.	Lamiaceae	Zarrin giah, Zeravi	H	leaves, flowers	Sedative, analgesia, inflammatory, anti-bacterial, anti-septic, foot pain
<i>Echinophora platyloba</i> DC.	Apiaceae	Khosharizeh	S	aerial plant	Anti fungal, spice and culinary
<i>Heracleum lasiopetalum</i> Boiss	Apiaceae	Goolpar, Kereson	H	fruit	Anti-septic, spice and condiment
<i>Kelussia odoratissima</i> Mozaff.	Apiaceae	Kelus, Bakhtyari karafs	H	leaves	Edible as vegetable, flavoring, indigestion, rheumatism
<i>Mentha longifolia</i> (L.) Hudson.	Lamiaceae	Pooneh, Pineh	H	leaves, flowers	Edible as vegetable, flavoring, indigestion, cough
<i>Satureja bachtiarica</i> Bung.	Lamiaceae	Marzeh Koohi	H	leaves, flowers	Edible as vegetable, flavoring, indigestion, cough, anti-bacterial
<i>Stachys lavandulifolia</i> Vahl.	Lamiaceae	Lolopashmak, Chaye Koohi	H	leaves, flowers	Green tea, anti-bacterial, skin diseases, menorrhagia
<i>Thymus daenensis</i> Celak.	Lamiaceae	Oushon, Avishan	H	leaves, flowers	Green tea, spice, culinary, cough, anti-bacterial, carminative
<i>Ziziphora tenuior</i> L.	Lamiaceae	Kakouti	H	leaves, flowers	Green tea, spice, culinary, anti-bacterial, carminative, anti-asthmatic

*Habit: T: Tree, S: Shrub, H: Herb

Table 2. Result of antibacterial tests of the investigated plants in agar diffusion assay (100 µg/disc)

Plant species	Extraction	^a <i>E.c</i>	^a <i>S.a</i>	^a <i>P.a</i>	^a <i>K.p</i>	b
<i>Heracleum lasiopetalum</i> Boiss	Ethanol extract	18	9	11	13	FR
	Essential oil	17	12	15	14	
<i>Saturja bachtiarica</i> Bunge.	Ethanol extract	22	14	13	12	LE
	Essential oil	23	21	22	14	
<i>Thymus daenensis</i> Celak	Ethanol extract	16	8	16	14	FL
	Essential oil	18	22	17	19	
<i>Ziziphora tenuir</i> L.	Ethanol extract	18	11	-	8	LE
<i>Echiophora platyloba</i> L.	Ethanol extract	12	9	10	16	ST
<i>Dracocephalam multicaule</i> Benth	Ethanol extract	22	-	-	12	SE
<i>Kelussia odoretascima</i> Mozff	Ethanol extract	10	-	12	10	LE
	Essential oil	16	9	16	17	
<i>Mentha longifolia</i> Hudson.	Ethanol extract	14	10	12	9	FL
	Essential oil	17	14	16	12	
<i>Achillea kellalensis</i> Boiss.	Ethanol extract	21	11	10	9	FL
	Essential oil	18	17	12	13	
<i>Stachys lavandulifolia</i> Vahl.	Ethanol extract	12	13	-	14	AP

a: Diameter of inhibition zone in mm.

b: part used (organ tested): FL: flower; FR: fruit; LE: leaves; ST: stem; SE: seed; AP: arial parts.

E.c: *Escherichia coli* ; P.a: *Pseudomonas aeruginosa*; S.a: *Staphylococcus aureu*; K..b: *Klebsiella pneumoniae*.

- : no inhibition

Table 3. Effect of extract and essential oil on growth bacteria strains by serial dilution assay (10 mg/ml)

Plant species	Growth inhabitation (%)				
	Extraction	<i>E.c</i>	<i>S.a</i>	<i>P.a.</i>	<i>K.p.</i>
<i>Heracleum lasiopetalum</i> Boiss.	Ethanol extract	70.66	37.30	57.80	55.31
	Essential oil	67.38	37.52	79.94	64.92
<i>Saturja bachtiarica</i> Bunge.	Ethanol extract	77.58	49.13	56.61	52.89
	Essential oil	76.30	66.23	73.22	67.82
<i>Thymus daenensis</i> Celak.	Ethanol extract	67.02	21.39	61.53	59.61
	Essential oil	74.55	62.46	77.70	71.39
<i>Ziziphora tenuir</i> L.	Ethanol extract	57.48	33.65	30.19	36.90
<i>Echiophora platyloba</i> L.	Ethanol extract	44.43	39.91	54.32	64.92
<i>Dracocephalam multicaule</i> Benth.	Ethanol extract	70.59	19.34	29.04	50.33
<i>Kelussia odoretascima</i> Mozff.	Ethanol extract	44.43	38.16	51.68	51.37
	Essential oil	65.64	32.25	69.10	69.99
<i>Mentha longifolia</i> Hudson.	Ethanol extract	61.94	26.66	48.15	45.98
	Essential oil	66.81	50.53	64.77	57.62
<i>Achillea kellalensis</i> Boiss.	Ethanol extract	73.38	28.70	62.78	49.75
	Essential oil	68.71	41.28	60.74	62.89
<i>Stachys lavandulifolia</i> Vahl.	Ethanol extract	41.53	46.12	35.57	63.28

E.c: *Escherichia coli* ; P.a: *Pseudomonas aeruginosa*; S.a: *Staphylococcus aureus*; K..b: *Klebsiella pneumoniae*.

Table 4. Minimal inhibitory concentration (MIC > 50%) for extracts and essential oils (MIC, µg/ml)

Plant species	Growth inhabitation (%)				
	Extraction	<i>E.c</i>	<i>S.a</i>	<i>P.a.</i>	<i>K.p.</i>
<i>Heracleum lasiopetalum</i> Boiss.	Ethanol extract	156.25	>1000	>1000	>1000
	Essential oil	39	>1000	156.25	39
<i>Saturja bachtiarica</i> Bunge.	Ethanol extract	156.25	>1000	625	>1000
	Essential oil	39	625	625	39
<i>Thymus daenensis</i> Celak.	Ethanol extract	156.25	>1000	39	625
	Essential oil	39	>1000	39	39
<i>Ziziphora tenuir</i> L.	Ethanol extract	625	>1000	>1000	>1000
<i>Echiophora platyloba</i> L.	Ethanol extract	>1000	>1000	156.25	156.25
<i>Dracocephalam multicaule</i> Benth.	Ethanol extract	625	>1000	>1000	>1000
<i>Kelussia odoretascima</i> Mozff.	Ethanol extract	>1000	>1000	>1000	>1000
	Essential oil	39	>1000	156.25	156.25
<i>Mentha longifolia</i> Hudson.	Ethanol extract	>1000	>1000	>1000	>1000
	Essential oil	156.25	>1000	625	625
<i>Achillea kellalensis</i> Boiss.	Ethanol extract	39	>1000	62	>1000
	Essential oil	39	>1000	625	625
<i>Stachys lavandulifolia</i> Vahl.	Ethanol extract	>1000	>1000	>1000	625

E.c: *Escherichia coli* ; *P.a*: *Pseudomonas aeruginosa*; *S.a*: *Staphylococcus aureu*; *K..b*: *Klebsiella pneumoniae*.

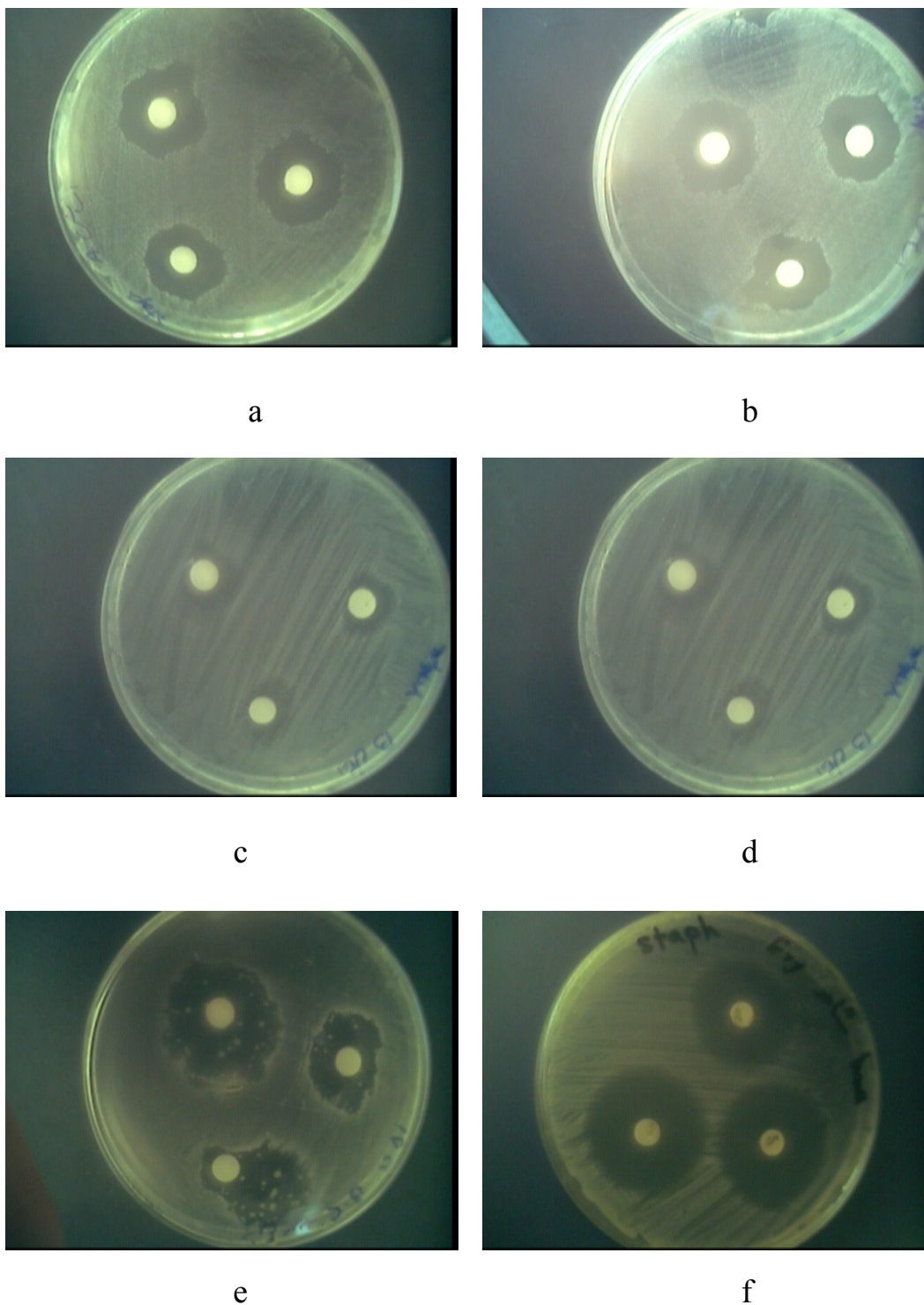


Figure 1. Sterile paper discs (6 mm in diameter) were impregnated with 60 µl of dilutions of known extract concentrations (100 µg/disc). Growth inhibition was measured and is expressed in millimeters.

a: Effect essential oil of *S. bachtiarica* on *S. aureus*, b: *T. daenensis* on *S. aureus*, c: *A. kellalensis* on *P. aeruginosa*, d: *M. longifolia* on *S. aureus*, e: *S. bachtiarica* on *E. coli*, f: Erythromycin on *S. aureus*