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 teniur* 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 posses' 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 teniur*, *Echiophora platyloba*, *Dracocephalam 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 powered (200g) and subjected to hydro-distillation (2000 ml distillated 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 power (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 10^7CFU/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 determinated 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 suspention at a density of 10^7 CFU/ml and incubated at 37° C for 48 h. The growth of microorganisms was observed as turbidity determinated 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):

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 essentiol oils could effectively inhibit the growth of *Eschershia coli*. Among these, the extracts of *Satureja bachtiarica*, *Dracocephalam multicule* and *Achillea kellalensis* 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. kellalensis* showed promising antibacterial activities against *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* (Table 4). The MIC (>50% growth inhabitation) 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 multifora* Boiss.) used in Iranian traditional medicine were investigated against some pathogenic food-borne bacteria. The minimum inhibitory concentrations of *Rhus coriaria* and *Zataria multifora* 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 erioealyx* 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 avtive constituents and evaluate the antibacterial activities against a wide rang of bacteria population.

References

Aktug, S. E & Karapinar, M. (1986). Sensitivity of some common food poisoning bacteria to thyme, mint and bay leaves. *International Journal of Food Microbiology, 3*: 349–354.

Arora, D. S., & Kaur, J. (1999). Antimicrobial activity of spices. *International Journal of Antimicrobial Agents, 12*: 257–262.

British pharmacopoeia. (1988). (Vol. 2, pp. 137–138). London: HMSO.

Cos, P., Vlietinck, A.J., Vanden Berghe, D., & Maes, L. (2006). Anti-infective potential of natural products: how to develop a stronger in vitro 'proof-of concept'. *Journal of Ethnopharmacology*, 106: 290–302.

Delgado, B., Palop, A., Fernandez, P. S., & Periago, P. M. (2004). Combined effect of thymol and cymene to control the growth of *Bacillus cereus* vegetative cells. *European Food Research and Technology, 218*: 188–193.

El-Khateib, T., & Abd El-Rahman, H. (1987). Effect of garlic and *Lactobacillus plantarum* on growth of *Salmonella typhimurium* in Egyptian fresh sausage and beef burger. *Journal of Food Protection, 50*: 310–311.

Fazeli, M.R., Amin, GH.R., Ahmadian Attari, M.M., Ashtiani, H., Jamalifar., H., & Samadi, N. (2007). Antimicrobial activities of Iranian sumac and avishan-e shirazi (*Zataria multiflora*) against some food-borne bacteria. *Food Control*, 18: 646–649.

Ghasemi Pirbalouti A. (2009). Medicinal plants used in Chaharmahal and Bakhtyari districts, Iran. *Herba Polonica,* 55: 34-38.

Ghasemi Pirbalouti, A., Roshan Chaleshtori, A., Tajbakhsh, E., Momtaz, H., Rahimi, E., & Shahin, F. (2009). Bioactivity of medicinal plants extracts against *Listeria monocytogenes* isolated from food. *Journal of Food Agriculture & Environment,* 7: 132-135.

Iennette, E.H. (1985). *Manual of clinical Microbiology fourth ed. American Association for Microbiology*, Washington, DC, pp. 978-987.

Kelmanson, J.E., Jager, A.K., & Van Staden, J. (2000). Zulu medicinal plants with antibacterial activity. *Journal of Ethnopharmacology*, 69: 241-246.

Nasar-Abbas, S. M., & Kadir Halkman, A. (2004). Antimicrobial effect of water extract of sumac (*Rhus coriaria* L.) on the growth of some food borne bacteria including pathogens. *International Journal of Food Microbiology,* 97: 63–69.

Ozcan, M., & Erkmen, O. (2001). Antimicrobial activity of the essential oils of Turkish plant spices. *European Food Research and Technology, 212*: 658–660.

Rasooli, I., Rezaei, M. B. & Allameh, A. (2006). Ultrastructural studies on antimicrobial efficacy of thyme essential oils on *Listeria monocytogenes*. *International Journal of Infection Diseases*, 10: 236-241.

Shelef, L. A. (1983). Antimicrobial effect of spices. *Journal of Food Safety, 6*: 29–44.

Srinivasan, D., Sangeetha, N., Suresh, T., & Perumalsamy, P.L. (2001). Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. *Journal of Ethnopharmacology,* 74: 217–220.

Zakaria, M., (1991). Isolation and characterization of active compounds from medicinal plants. *Asia Pacific Journal Pharmacology*, 6: 15–20.

Zampini, I. C., Vattuone, M.A. and & Isla, M. I. (2005). Antibacterial activity of *Zuccagnia punctata* Cav. ethanolic extracts. *Journal of Ethnopharmacology*, 102: 450–456.

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

Scientific name	**Family name**	**Local name**	**Habit***	**Parts used**	**Uses/ailments treated**
Achillea kellalensis Boiss. & Hausskn.	Asteraceae	Golberenjaz	H	flowers	Wound, carminative, indigestion
Dracocephalum multicaule Montbr & Auch.	Lamiaceae	Zarrin giah, Zeravi	H	leaves, flowers	Sedative, analgesia, inflammatory, anti-bacterial, anti-septic, foot pain
Echinophora platyloba DC.	Apiaceae	Khosharizeh	S	aerial plant	Anti fungal, spice and culinary
Heracleum lasiopetalum Boiss	Apiaceae	Goolpar, Kereson	H	fruit	Anti-septic, spice and condiment
Kelussia odoratissima Mozaff.	Apiaceae	Kelus, Bakhtyari karafs	H	leaves	Edible as vegetable, flavoring, indigestion, rheumatism
Mentha longifolia (L.) Hudson.	Lamiaceae	Pooneh, Pineh	H	leaves, flowers	Edible as vegetable, flavoring, indigestion, cough
Satureja bachtiarica Bung.	Lamiaceae	Marzeh Koohi	H	leaves, flowers	Edible as vegetable, flavoring, indigestion, cough, anti-bacterial
Stachys lavandulifolia Vahl.	Lamiaceae	Lolopashmak, Chaye Koohi	H	leaves, flowers	Green tea, anti-bacterial, skin diseases, menorrhagia
Thymus daenensis Celak.	Lamiaceae	Oushon, Avishan	H	leaves, flowers	Green tea, spice, culinary, cough, anti-bacterial, carminative
Ziziphora tenuior 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]*E.c***	**[a]*S.a***	**[a]*P.a***	**[a]*K.p***	**b**
Heracleum lasiopetalum Boiss	Ethanol extract	**18**	9	11	13	
	Essential oil	**17**	12	15	14	FR
Saturja bachtiarica Bunge.	Ethanol extract	**22**	14	13	12	
	Essential oil	**23**	**21**	**22**	14	LE
Thymus daenensis Celak	Ethanol extract	**16**	8	16	14	
	Essential oil	**18**	**22**	17	**19**	FL
Ziziphora teniur L.	Ethanol extract	**18**	11	**-**	8	LE
Echiophora platyloba L.	Ethanol extract	12	9	10	16	ST
Dracocephalam multicaule Benth	Ethanol extract	**22**	**-**	**-**	12	SE
Kelussia odoretascima Mozff	Ethanol extract	10	**-**	12	10	
	Essential oil	**16**	9	16	**17**	LE
Mentha longifolia Hudson.	Ethanol extract	14	10	12	9	
	Essential oil	**17**	14	16	12	FL
Achillea kellalensis Boiss.	Ethanol extract	**21**	11	10	9	
	Essential oil	**18**	**17**	12	13	FL
Stachys lavandulifolia 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

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

Plant species	**Growth inhabitation (%)**				
	Extraction	***E.c***	***S.a***	***P.a.***	***K.p.***
Heracleum lasiopetalum Boiss.	Ethanol extract	**70.66**	37.30	57.80	55.31
	Essential oil	**67.38**	37.52	**79.94**	**64.92**
Saturja bachtiarica Bunge.	Ethanol extract	**77.58**	49.13	56.61	52.89
	Essential oil	**76.30**	**66.23**	**73.22**	**67.82**
Thymus daenensis Celak.	Ethanol extract	**67.02**	21.39	**61.53**	59.61
	Essential oil	**74.55**	**62.46**	**77.70**	**71.39**
Ziziphora teniur L.	Ethanol extract	57.48	33.65	30.19	36.90
Echiophora platyloba L.	Ethanol extract	44.43	39.91	54.32	**64.92**
Dracocephalam multicaule Benth.	Ethanol extract	**70.59**	19.34	29.04	50.33
Kelussia odoretascima Mozff.	Ethanol extract	44.43	38.16	51.68	51.37
	Essential oil	**65.64**	32.25	**69.10**	**69.99**
Mentha longifolia Hudson.	Ethanol extract	**61.94**	26.66	48.15	45.98
	Essential oil	**66.81**	50.53	**64.77**	57.62
Achillea kellalensis Boiss.	Ethanol extract	**73.38**	28.70	**62.78**	49.75
	Essential oil	**68.71**	41.28	**60.74**	**62.89**
Stachys lavandulifolia Vahl.	Ethanol extract	41.53	46.12	35. 57	**63.28**

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

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

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

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

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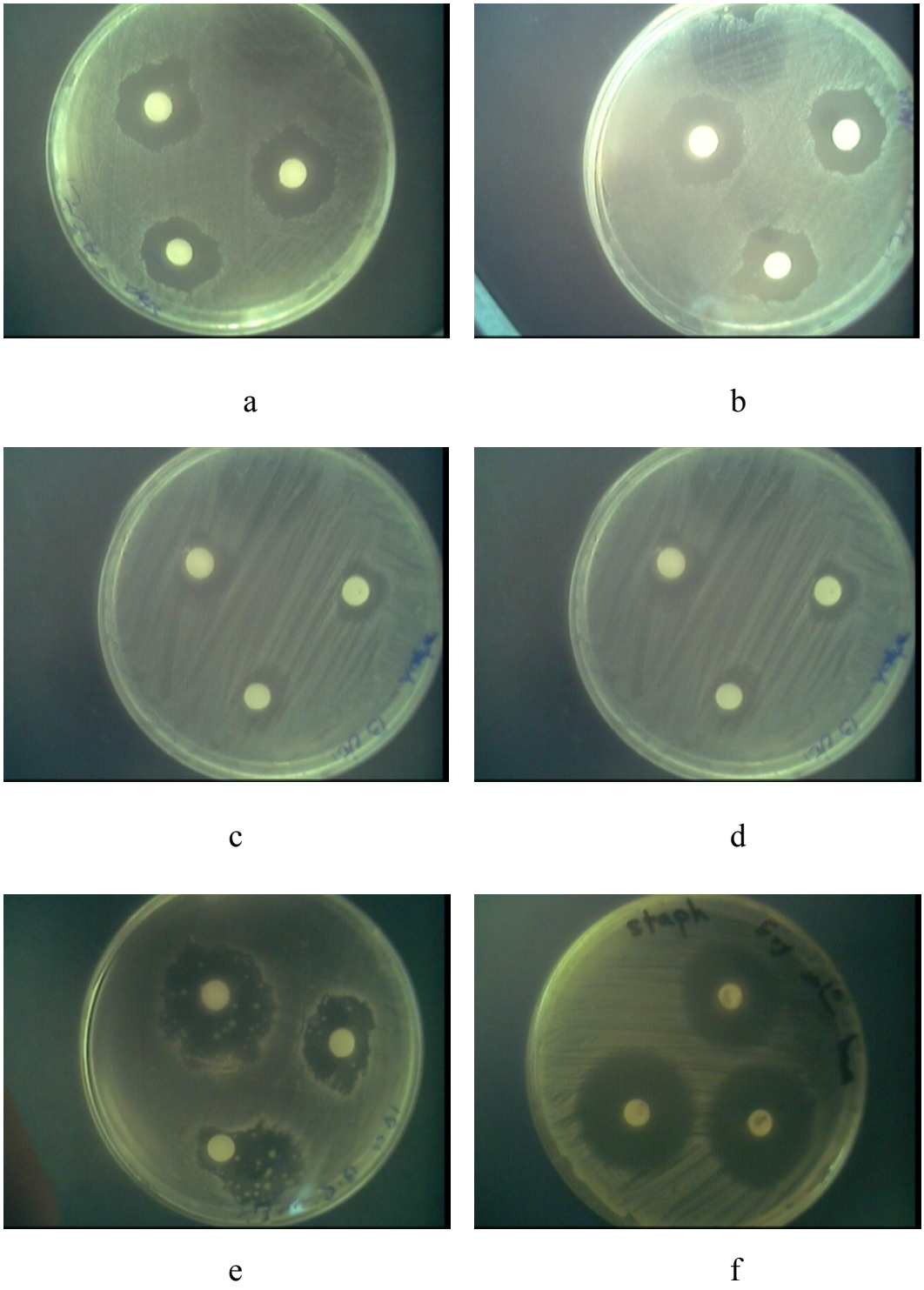

Figure 1. Sterile paper discs (6 mm in diameter) were impregnated with 60 µl of dilutions of known extract concentrations (100 µg/disc). Growth inhibition was measured and is expressed in millimeters.

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*