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Antimicrobial and Antioxidant Activities of Some Medicinal Smokes Prescribed in Iranian Traditional Medicine for Catarrh

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Abstract

Background: Microbial resistance has recently become one of the major healthcare problems worldwide. Finding new sources of chemical active antimicrobial compounds, along with what has been produced by fungi is now a common approach to solve this problem. On the other hand, inflammation has recently been considered as an underlying factor of many diseases, and working on anti-inflammatory drugs is interesting as well. Information provided by Iranian traditional medicine (ITM) can be used as a source of inspiration in order to find the herbs with antimicrobial and antioxidant activity.

Objective: The present study aimed to examine antimicrobial and antioxidant activities of 6 medicinal smokes prescribed in ITM for catarrh.

Materials and Methods: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, *Bacillus subtilis* ATCC 6051, *Candida albicans* ATCC 10231, and *Aspergillus niger* ATCC 16404 were used in this study. In addition, the preliminary antimicrobial activity of *Commiphora myrrha*, *Cinnamomum cassia*, *Costus arabicus*, *Nigella sativa*, *Pimpinella anisum*, and *Tetraclinis articulate* was performed by the disc diffusion method. Further, the minimum inhibitory concentration and minimum lethal concentration were determined using the microdilution method. Finally, antioxidant activity was evaluated by measuring the reduction of the 1,1-diphenyl-2-picrylhydrazyl solution.

Results: The results showed that *C. arabicus*, *C. cassia*, and *P. anisum* had considerable antimicrobial activities. Furthermore, antioxidant evaluation of the herbal smokes demonstrated the lowest IC50 for *C. cassia* (10.44 µg/mL), *C. myrrha* (13 µg/mL), and *C. arabicus* (15.16 µg/mL).

Conclusion: In general, herbal medicinal smokes were found to have interesting antimicrobial and antioxidant activities. Therefore, more investigation on these smokes would be fruitful.

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Background

Nowadays, microbial resistance is considered as one of the main healthcare problems around the world.[1] Therefore, investigating new sources of chemical active antimicrobial compounds, as well as what is produced by the fungi and exist in the shelf as antibiotics is now highlighted as a common method for solving such problem.[2] In addition, inflammation is regarded as an underlying determinant of various diseases, and studying anti-inflammatory drugs seems interesting.[3] Plants are considered as a huge pool of chemical compounds, especially the large and sophisticated one.[4] Therefore, plants would be a good candidate for developing antimicrobial and anti-inflammatory agents.

In Iranian traditional medicine (ITM), many infectious and inflammatory diseases have been well-described and thus numerous simple and compound drugs have been prescribed for their treatment. These treatments can provide valuable clues to initiate drug discovery investigation. Catarrh, which is traditionally called "Zokam",[5] is associated with the common cold, pharyngitis, and chesty coughs. All these signs are common in inflammation of the mucous membranes and can result in infection. ITM prescribes medicinal smokes to deliver active compounds of herbal medicines to respiratory tracts. Previously, different studies investigated antimicrobial activities of medicinal smokes,[6-8] some of which have gone forward and isolated

active antibacterial components.[6] However, studying anti-inflammatory components of medicinal smoke has been rare.[6] Therefore, the current study sought to investigate the antimicrobial and anti-inflammatory activities of some medicinal smokes traditionally prescribed for Zokam.

Materials and Methods

Selection and Preparation of Medicinal Plants

In order to study the traditional prescription for Zokam, ITM prominent books including *Al-Hawi* (Rhazes 865-925 A.D.), *Canon of Medicine* (Avicenna 970-1051 A.D.), *Zakhire-ye-Khawrazmshahi* (Jorjani 12th century A.D.), *Makhzan ul-Advia (Aqili Khorasani* 18th century A.D.), and *Exir-e-A'zam* (Chishti 18th century A.D.) were studied.[9-13] Based on the frequency of use, seven of the most useful herbs, namely, *Commiphora myrrha*, *Cinnamomum cassia*, *Costus arabicus*, *Nigella sativa*, *Pimpinella anisum*, and *Tetraclinis articulate* were selected for the purpose of the study. The plants were prepared from Tehran botanical market and authenticated by Dr. Yousef Ajeni in the Herbarium of Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran. Then, the plant materials were powdered and maintained at 25°C until use.

Smoke Extraction

About 200 g of each of the dried plants was separately powdered and filled up in a round-bottom flask in order to prepare the smoke extract. Then, the flask was heated through a heating mantle to burn the powdered plant. Next, the raised smoke was collected by a distillation set to another flask filled with methanol. Finally, the yield of extraction (w/w) was calculated as the weight of dry extract/weight of dry starting material ×100.

Microbial Strains and Preliminary Antimicrobial Evaluations of Smoke

Staphylococcus aureus ATCC 25923, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, *Bacillus subtilis* ATCC 6051, *Candida albicans* ATCC 10231, and *Aspergillus niger* ATCC 16404 were used based on the aim of the study. The microbial suspensions were prepared in normal saline with the concentration of 10^4 or 10^6 CFU/mL for bacteria and fungi, respectively, using standard routine spectrophotometric methods. Further, the antimicrobial activities were determined by disc diffusion and broth microdilution assays. In the disc diffusion method, the above standard microbial suspensions were loaded on Mueller Hinton agar and Sabouraud dextrose agar by a cotton swab for bacteria and fungi, respectively. Then, sterile paper discs (6.8 mm) were impregnated with 0.2 mg of each smoking extract and placed on the culture plates. Next, vancomycin (30 µg/disc), gentamycin (10 µg/disc), and amphotericin B (10 µg/disc), provided from Padtan Teb, Iran, were used as positive control. Furthermore, the diameters of inhibition zones (mm) around the disks were measured after overnight incubation. All experiments were conducted in triplicate.[14]

The Minimum Inhibitory Concentration and Minimum Lethal Concentration Determination

The minimum inhibitory concentration (MIC) and Minimum Lethal Concentration (MLC) values were determined in broth microdilution assay. Moreover, the smoking extracts were twofold serially diluted in pure methanol, which contained 10-0.156 mg/mL. Antibiotic powders (i.e., gentamicin, vancomycin, and amphotericin B) were used as positive control. These dilutions were prepared in a 96-well microtiter plate. Additionally, morpholinepropanesulfonic acid-buffered RPMI 1640 and Mueller-Hinton broth were utilized as broth media for fungi and bacteria, respectively. After shaking, 100 µL of extract dilutions was added to each well. Then, 100 µL of diluted microbial suspensions (1×10^6 CFU/mL for bacteria and 10^4 CFU/mL for fungi) were added to each well and incubated at suitable conditions. MICs were defined as the lowest concentration of dilution that inhibits bacteria and fungi after 24 and 48 hours, respectively. Finally, MLC values were the first well that showed no growth on solid media.[15]

Antioxidant Evaluation by the Reduction of DPPH Solution

Different concentrations of smoking extracts in methanol (0.007-2 mg/mL) were mixed with 2 mL of 1,1-diphenyl-2-picrylhydrazyl (DPPH) solution. The solutions were incubated at room temperature for 70 minutes. Then, the absorbance of each solution against the blank was determined at 517 nm. Next, the inhibition of free radicals by DPPH in percent (I%) was calculated in the following way:

$$I\% = [A_{blank} - A_{sample}/A_{blank}] \times 100$$

Butylated hydroxytoluene was used as positive control (16).

Results

Selection of Plants

Table 1 presents the most frequent herbs prescribed in the form of the smoke for Zokam based on ITM books. Persian and English common names, part used, and the yield of extraction are provided as well.

Antimicrobial Evaluations

Evaluating the smokes by disc diffusion method exhibited that gram-positive bacteria had higher inhibition zone diameters compared to gram-negative bacteria, yeast, and mold. Among 2 gram-negative bacteria, the inhibition zone diameters for *E. coli* were higher than those for *P.*

Table 1. Most Frequent Herbs Prescribed in ITM in the Form of the Smoke for Zokam

Scientific Names	Persian Common Names	Part Used	Yield of Smoke Extraction (%)
Commiphora myrrha	Morr	Oleogum resin	3
Cinnamomum cassia	Salikha	Bark	6.43
Costus arabicus	Qost	Root	6.5
Nigella sativa	Siahdaneh	Seed	8.1
Pimpinella anisum	Anisoon	Fruit	4.5
Tetraclinis articulate	Sandoroos	Resin	4.3

Abbreviations: ITM, Iranian traditional medicine.

aeruginosa. In addition, *C. albicans* had an inhibition zone for some smokes. However, no inhibition zone for smokes was observed against *P. aeruginosa* and *A. niger*. Among different smokes, *C. myrrha* had higher inhibition zone diameters for *S. aureus*, *B. subtilis*, *E. coli*, and *C. albicans*, followed by *P. anisum* while *N. sativa. C. cassia,* and *C. arabicus* demonstrated inhibition zones only for *S. aureus* and *B. subtilis*. Further, *T. articulate* showed an inhibition zone diameter for *B. subtilis* and *E. coli* (Table 2).

The results of broth microdilution for herbal smokes varied from the disc diffusion methods. The difference can be as the result of the behavior of smokes in solid or broth media. Furthermore, the results indicated that all the smokes revealed considerable antimicrobial activities except for *N. sativa* and *T. articulate* (Table 3).

Moreover, the antioxidant evaluation of the herbal smokes showed the lowest IC_{50} for *C. myrrha* (13 µg/mL), *C. cassia* (10.44 µg/mL), and *C. arabicus* (15.16 µg/mL), followed by *P. anisum* (57.6 µg/mL) and *N. sativa* (215.7 µg/mL) while higher IC_{50} was for *T. articulate* (262.9 µg/mL). The IC_{50} for Butylated hydroxytoluene as the positive control was 19 µg/mL (Table 3).

Discussion

As previously mentioned, medicinal smokes have been recently recognized as one of the interesting topics in the field of natural antimicrobials. Mohagheghzadeh et al in a review article explained traditional utilization of medicinal smokes in different civilizations and clarified that medicinal smokes have long been used against many ailments with infectious etiology. Air purification and disinfectant are to examples of traditional utilization of medicinal smokes. Additionally, different infectious diseases including pulmonary conditions were traditionally targeted by medicinal smokes.[17]

Experimental investigations have been recently implemented and antibacterial activities of the smokes of many plants such as *Etlingera brevilabrum*, *Santalum album*, *Cymbopogon schoenanthus*, *Boswellia serata*, *Ficus religiosa*, *Mangifera indica*, and the like were evaluated.[6-8] Shahverdi et al in their study clarified the antibacterial activity of *Peganum harmala* smoke (Esfand), the smoke traditionally used by Iranians as an air purifier, on both Gram-positive and Gram-negative bacteria, along with fungi.[18] In another study published on the air purification effect of the smoke of an Indian polyherbal mixture Havan Samagri, a sacred offering mentioned in Vadas, airborne bacteria reduced up to 94% by 60 minutes.[19]

As regards the differences of essential oil and smoke constituents of the essential oil-bearing herbs, the results of another study showed that chemical constituents of the essential oil and the smoke of *Pistacia terebinthus* oleo gum resin was different in both chemical constituents and the ratio of the compounds.[20] It means that burning procedure changes the active components and consequently, alters the activity of medicinal plants. In this case, minimum inhibitory concentrations of the smoke against gram-positive and gram-negative bacteria and fungi were surprisingly in the range of 1.5 to 6.25 µg/mL.[20]

In this study, it was attempted to select the herbs by the

Table 2. The Antimicrobial Evaluation of Herbal Smokes by Disc Diffusion Method

Herbal Smoke	Inhibition Zone Diameter (mm)					
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. niger*
C. arabicus	8.7	10.9	-	-	-	-
C. cassia	9.1	12.2	-	-	-	-
C. myrrha	14.4	12.8	9.7	-	7.9	-
N. sativa	8.4	9.5	7.5	-	9.3	-
P. anisum	9.3	13.2	8.4	-	7.5	-
T. articulate	-	10.1	10.1	-	-	-
Amphotericin B	ND	ND	ND	ND	24.6	16.9
Gentamicin	ND	31.7	21.9	18.3	ND	ND
Vancomycin	16.6	ND	ND	ND	ND	ND

Note. (-): No inhibition zone; ND: Disc diffusion.

Table 3. The Antimicrobial and Antioxidant Evaluation of Herbal Smokes by Broth Dilution and DPPH Reduction Assay

	S. aureus		*B. subtilis*		*E. coli*		*P. aeruginosa*		*C. albicans*		*A. niger*		Antioxidant Evaluation (µg/mL)
	MIC	MLC	MIC	MLC	MIC	MLC	MIC	MLC	MIC	MLC	MIC	MLC	
C. arabicus	0.39	0.81	1.6	3.2	0.81	1.6	0.8	0.8	0.39	0.8	0.39	0.8	15.16
C. cassia	0.17	0.34	0.72	1.4	0.72	1.4	1.4	1.4	0.72	1.4	0.17	0.34	10.44
C. myrrha	0.39	0.78	0.39	0.39	>3.2	>3.2	0.39	0.78	0.39	0.78	0.78	0.78	13
N. sativa	0.51	0.51	0.26	0.26	>2.1	>2.1	>2.1	>2.1	>2.1	>2.1	>2.1	>2.1	215.7
P. anisum	0.22	0.45	0.22	0.22	>0.9	>0.9	0.22	0.22	0.11	>0.9	0.45	>0.9	57.6
T. articulate	0.19	0.38	0.09	0.09	>0.8	>0.8	>0.8	>0.8	>0.8	>0.8	>0.8	>0.8	262.9
Vancomycin	0.5	1	-	-	-	-	-	-	-	-	-	-	-
Gentamicin	-	-	0.5	1	2	4	2	4	-	-	-	-	-
Amphotericin B	-	-	-	-	-	-	-	-	0.5	1	1	2	-
BHT													19

MIC; Minimal inhibitory concentration; MLC: Minimal lethal concentration; DPPH: 1,1-diphenyl-2-picrylhydrazyl; BHT: Butylated hydroxytoluene.
Note. The MIC and MLC for smoke and antibiotic were based on mg/mL and µg/mL, respectively; (-): Not applicable.

aid of ITM. The goal was to find medicinal smokes with dual antimicrobial and antioxidant activity. Accordingly, a total of 6 herbs were selected and their smoke was prescribed for Zokam, which is a traditional ailment with inflammatory and microbial etiology. The results of the current study revealed that the smokes of *C. myrrha*, *C. cassia*, and *C. arabicus* had strong antioxidant activity even stronger than butylated hydroxytoluene. In addition, the smokes of these herbs had interesting antimicrobial activities against gram-negative and gram-positive bacteria, as well as fungi. Although there are some investigations on antimicrobial activities of the plant parts and essential oils of *C. myrrha*, *C. cassia*, and *C. arabicus*,[21-23] few studies have been conducted concerning the smoke of these herbs. Therefore, similar studies focusing on these smokes are subject to further investigation.

Conclusion

In general, medicinal smokes are frequently used in ITM as a therapeutic agent. Based on the results of the present study, *C. myrrha*, *C. cassia*, and *C. arabicus* smoke extracts had strong antioxidant and antimicrobial activities. Finally, more investigation on this field would be fruitful since studies on the therapeutic effects of medicinal smokes are highly limited.

Authors' Contributions

All authors participated equally in the present study.

Ethical Approval

Not applicable.

Conflict of Interest Disclosures

The authors declare that they have no conflict of interests.

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References

1. Zaman SB, Hussain MA, Nye R, Mehta V, Mamun KT, Hossain N. A review on antibiotic resistance: alarm bells are ringing. Cureus. 2017;9(6):e1403. doi:10.7759/cureus.1403
2. Durand GA, Raoult D, Dubourg G. Antibiotic discovery: history, methods and perspectives. Int J Antimicrob Agents. 2019;53(4):371-382. doi:10.1016/j.ijantimicag.2018.11.010
3. Hunter P. The inflammation theory of disease. The growing realization that chronic inflammation is crucial in many diseases opens new avenues for treatment. EMBO Rep. 2012;13(11):968-970. doi:10.1038/embor.2012.142
4. Mishra BB, Tiwari VK. Natural products: an evolving role in future drug discovery. Eur J Med Chem. 2011;46(10):4769-4807. doi:10.1016/j.ejmech.2011.07.057
5. Shirzad M, Iran-Nejad S, Cheraqi Niroumand M, Shams Ardekani MR. Iranian Traditional Medicine: A Dictionary. Tehran: Traditional Medicine and Materia Medica Research Center; 2014.
6. Mahdavi B, Yaacob WA, Din LB. Phytochemical study of medicinal smokes from *Etlingera brevilabrum* leaves. J Herb Med. 2018;13:52-62. doi:10.1016/j.hermed.2018.04.001
7. Samanth T, Jha SG, Sinha V, Patel S, Desai K. Effect of smoke from medicinal herbs on the nosocomial infections in ENT outpatient department. Indian J Otol. 2018;24(1):9-12. doi:10.4103/indianjotol.INDIANJOTOL_143_16
8. Khosravian Dehkordi F, Jamalifar H, Amin GR, et al. Antimicrobial effect of some medicinal smoke, Iranian traditional medicine. J Med Plants. 2017;1(16):185-196.
9. Rhazes (865- 925 A.D.). Al-Hawi. Beirut: Dar ul-Ehya At-Turath al-Arabi; 2001.
10. Avicenna (970- 1051 A.D.). Ghanoon (Canon of Medicine). Translated by A. Sharafkandi. Tehran: Soroush Press; 2010.
11. Jorjani SE (12th century A.D.). Zakhire-ye-Khawrazmshahi. Tehran: Bonyad-e Frahange Iran; 1976.
12. Aqili Khorasani MH (18th century A.D.). Makhzan ul-Advia. Tehran: Enqelab-e Eslami Publishing and Educational Organization; 1992.
13. Chishti MA (18th century A.D.). Exir-e-A'zam. Tehran: Research Institute for Islamic and Complementary Medicine; 2004.

14. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Disc Susceptibility Test. 11th ed. Wayne, Pennsylvania: CLSI; 2012.
15. Clinical and Laboratory Standards Institute (CLSI). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. 8th ed. Wayne, Pennsylvania: CLSI; 2009.
16. Burits M, Bucar F. Antioxidant activity of *Nigella sativa* essential oil. Phytother Res. 2000;14(5):323-328.
17. Mohagheghzadeh A, Faridi P, Shams-Ardakani M, Ghasemi Y. Medicinal smokes. J Ethnopharmacol. 2006;108(2):161-184. doi:10.1016/j.jep.2006.09.005
18. Shahverdi AR, Monsef-Esfahani HR, Nickavar B, Bitarafan L, Khodaee S, Khoshakhlagh N. Antimicrobial activity and main chemical composition of two smoke condensates from Peganum harmala seeds. Z Naturforsch C. 2005;60(9-10):707-710.
19. Nautiyal CS, Chauhan PS, Nene YL. Medicinal smoke reduces airborne bacteria. J Ethnopharmacol. 2007;114(3):446-451. doi:10.1016/j.jep.2007.08.038
20. Mohagheghzadeh A, Faridi P, Ghasemi Y. Analysis of Mount Atlas mastic smoke: a potential food preservative. Fitoterapia. 2010;81(6):577-580. doi:10.1016/j.fitote.2010.01.022
21. Shuaib M, Ali A, Ali M, Panda BP, Ahmad MI. Antibacterial activity of resin rich plant extracts. J Pharm Bioallied Sci. 2013;5(4):265-269. doi:10.4103/0975-7406.120073
22. Ooi LS, Li Y, Kam SL, Wang H, Wong EY, Ooi VE. Antimicrobial activities of cinnamon oil and cinnamaldehyde from the Chinese medicinal herb *Cinnamomum cassia* Blume. Am J Chin Med. 2006;34(3):511-522. doi:10.1142/s0192415x06004041
23. Al-Musayeib NM, Mothana RA, Matheeussen A, Cos P, Maes L. In vitro antiplasmodial, antileishmanial and antitrypanosomal activities of selected medicinal plants used in the traditional Arabian Peninsular region. BMC Complement Altern Med. 2012;12(1):49. doi:10.1186/1472-6882-12-49