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Have the Egyptian Moâssel Products Antimicrobial Activities?

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Abstract

As a result of the mistaken belief of some people that Waterpipe has some positive effects to kill some microbes. That is why we did this study. The samples Mango Flavored Moâssel (MFM) and Peppermint Flavored Moâssel (PepFM) were obtained from Al Dandash company. They were analyzed by Headspace GC-MS. There was a great difference between the two investigated samples. The 21 identified compounds of the MFM sample were detected, which represented (88.85%) of the total compounds. The major one was 1,2-propanediol (41.40%). While, the PepFM specimen exhibited 26 identified compounds, which represented (76.84%) of the total constituents. The chief constituent was menthol (33.89%). MFM displayed 6 common compounds with PepFM; acetone, furfural, limonene, linalool, *α*-terpineol and carvone. The tested bacterial strains (*Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) were susceptible to growth inhibition by MFM and PepFM methanol condensates. Moreover, the tested fungus *Candida albicans* exhibited also growth inhibition by the two flavored methanol condensates. Finally, microbial adherence was achieved at higher concentrations of methanol condensates (above MIC). This is due to the growth inhibitory effects of methanol condensates. While, at low concentrations (sub MIC), it was found that microbial adherence increased. Consequently, these flavored Moâssel are not recommended to use them as antimicrobial agents.

Keywords
Waterpipe (Hookah); Mango Flavored Moâssel; Peppermint Flavored Moâssel; Headspace GC-MS; Antimicrobial; Microbial adherence.

1. Introduction

Waterpipe smoking is a sort of tobacco smoking. The most common type of tobacco used in the Waterpipe is called Moâssel, which is a mixture of crude fermented tobacco with molasses. The artificial mixtures of volatile flavor constituents are usually added to imitate the respective natural flavor and to cover the bitterness of tobacco smoke compared to cigarettes, make it more appealing to users [1]. Waterpipe (syn.: Hookah; Goza, Narghile, Arghile, Shisha and Hubble bubble) is a pipe used to smoke a combination of tobacco, which is either flavored or unflavored [2].

According to a WHO advisory, a typical one-hour session of Waterpipe smoking exposes the user to 100 to 200 times the volume of smoke inhaled from a single cigarette [3]. Waterpipe smoking is more harmful than cigarette smoking because even after the smoke passing through water vessel, it still contains high levels of the tobacco addictive substance “nicotine”, many toxic compounds such as carbon monoxide, heavy metals, carcinogens like tobacco specific nitrosamines, and different added Moâssel artificial flavoring substances [2].

Al Dandash company is one of the most famous companies producing many Moâssel products in Egypt. Literature survey, eight Egyptian flavored Moâssel samples from this company were chemically analyzed by Headspace GC-MS *viz*., Apple, Creamy Strawberry, Mix Grapes, Guava, Mixed Fruits, Watermelon, Peach and Kas [4- 6].

Pulmonary inflammation and increased oxidative stress of Peach and Kas Moâssel smoke were investigated. They demonstrated by increasing levels of NO and MDA as well as histopathological changes in rat lung tissues [6].

Little knowledge is available about the toxicological and antimicrobial impact of these added flavors after being burnt by the Waterpipe smokers. Such relation needs to be explored as it is crucial for the assessment of potential health hazards linked with these flavors.

Consequently, the current study designed to investigate the chemical constituents of more Waterpipe Moâssel products from Al Dandash company; “Mango Flavored Moâssel (MFM) and Peppermint Flavored Moâssel (PepFM)” for their volatile profile by Headspace gas chromatography-mass spectrometry (Headspace GC-MS). Moreover, it aimed to explore the antimicrobial activities of these Waterpipe Moâssel products.

2. Material and Methods

2.1. Moâssel products

Two flavored Moâssel products; Mango Flavored Moâssel (MFM) and Peppermint Flavored Moâssel (PepFM)” were obtained from Al Dandash company, Egypt.

2.2. Headspace GC/MS

The Moâssel products (MFM and PepFM) were subjected to Headspace GC-MS analysis. Shimadzu GC-MS with Headspace

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system provided by Flame Ionization Detector, connected to the Mass Spectrometer (MS) Model: QP2010Ultra. Total ion chromatograms (TIC) and mass spectra were recorded in the electron impact (EI) ionization mode at 70 eV, using ACQ Mode (scan from 35 to 500 m/z in 0.3 sec). The dimensions of the utilized column were 0.25 mm in internal diameter, 30 m length, packed with Rtx-MS and 0.25 μm film thickness. The volume of the injected was 1.0 μL, using helium as carrier gas (flow rate 40 mL/min). The Headspace GC-MS analysis was carried out at a programmed temperature; starting with the initial temperature was 40 °C (2 min), followed by an increased (rate 30-50 °C) to the final temperature 210 °C (5 min). Both of injector and detector had the same temperature 230 °C. The total run was 45 min and split ratio 1:50 [5-6].

2.3. Extraction of the methanol condensates of Moâssel products

The methanol condensates of the two flavored Moâssel products were obtained in the methanol trapped flasks after burning on Waterpipe by aiding of a vacuum pump [6].

2.4. Antimicrobial study

2.4.1. Microorganisms

Bacterial strains: Staphylococcus aureus (Gram-positive, Facultative anaerobic), *Escherichia coli* (Gram-negative, Facultative anaerobic), *Klebsiella pneumonia* (Gram-negative, Facultative anaerobic), *Pseudomonas aeruginosa* (Gram-negative, Facultative aerobic). *Fungal strain: Candida albicans* (Diploid fungus). The bacterial and fungal strains used in the study were clinical isolates obtained from Department of Microbiology and Immunology, Faculty of Pharmacy, Minia University, Egypt.

2.4.2. Determination of inhibition zone (IZ)

Preparation of the sample was performed by dissolving the methanol condensate (obtained from the condensation of the smoke in methanol during burning using a pump for suction of smoke) in DMSO to obtain the desired concentrations. For assessment of the antimicrobial activity using agar-well diffusion technique, the bacterial cultures were adjusted to 0.5 mL of 1 x 10^6 CFU/mL (0.5 Mcfarland turbidity) and the fungal cultures were adjusted to the concentration 1 x 10^6 CFU/mL according to Hamed et al., 2020 and El-Kashef et al., 2015 [7-8].

2.4.3. Determination of minimum inhibitory concentration (MIC)

The MIC values of the methanol condensates, antibiotics and antifungal were determined using two-fold serial dilution to prepare concentrations of 10, 5, 2.5 and 1.25 mg/mL according to Hamed et al., 2020 and El-Kashef et al., 2015 [7-8].

2.4.4. Adherence assay method (Tissue culture plate method, TCP)

Firstly, all strains were streaked into Trypticase soy agar then, incubated at 25 °C for 48 h. A large loop of actively growing cells (for each strain) was transferred to sterile Trypticase Soy Broth (TSB) (Difco Laboratory) containing 0.9% D-glucose. After incubation, the cells were centrifuged and washed twice with 0.5 mL PBS (Phosphate Buffered Saline), followed by vortex and centrifugation at 5000 g for 5 min.

The washed cells were suspended in 1 mL TSB broth and adjusted to the final OD600 nm value of 1.0 with TSB broth. These cell suspensions were then used to grow biofilms. One hundred μL of suspension (OD600) was inoculated into individual wells of polystyrene 96-well plates (flat bottom; Nunc).

The TSB broth was used as the negative control. The plates were incubated at 25 °C for 90 min (adhesion period). Supernatants including planktonic cells were discarded and well was gently washed with PBS twice to remove any non-adherent cells. One hundred μL of fresh TSB broth containing MIC or above MIC or sub-MIC concentrations of each of the methanol condensate was added to each well.

The plates were covered to prevent evaporation and incubated at 25 °C for 24 h. Liquid media containing the non-adherent cells were discarded through two rounds of washing with 200 μL sterile PBS buffer. Adherent cells to the plastic surfaces were quantified using the crystal violet assay. The experiment was performed in triplicate.

The TCP assay is considered as a standard test for the detection of biofilm formation and the ability of microorganisms to adhere to the plastic surface. All isolates were screened for their ability to adhere to the surface of tissue culture plate surface by the TCP method as described by Christensen et al., 1985 [9] with a modification in the duration of incubation which was extended to 24 h, according to O'Toole and Kolter 1998 [10].

3. Results and Discussion

Identification of the volatile components in MFM and PepFM was carried out by direct comparison of retention time (Figures 1 and 2) and fragmentation pattern of each of the identified compounds and quantitation was based on peak area integration [11]. The GC-MS identified compounds are listed in (Tables 1 and 2). The volatile profile of the MFM sample smoke contained twenty-one volatile compounds belonging to two major classes *viz.* oxygenated and hydrocarbons compounds totaling 88.44 and 00.41%, respectively (Table 1). On the other side, only twenty-six volatile compounds were identified in the PepFM mostly oxygenated compounds amounted to 67.10% of the identified compounds (Table 2).

Both chromatograms have six common peaks corresponding to acetone, furfural, limonene, linalool, α-terpineol and carvone.

In MFM, 1,2-propanediol (41.40%) was the major compound followed by *γ*-decalactone (11.83%), 5,6-epoxy-*β*-ionone (11.15%) and *z*-3-hexen-1-ol (9.18%). The undiluted 1,2-propanediol was minimally irritating to the eye and making slight transient conjunctivitis. It improved after the exposure removed [12].

Table 1: Identified compounds of MFM from Headspace GC-MS.

No.	Name	RT*	RRT**	Base peak	Relative Area %	M. Weight	M. Formula
1	5,6-Epoxy-*β*-ionone	1.46	0.350	40	11.15	208	$C_{13}H_{20}O_2$
2	Acetone	1.73	0.414	43	0.67	58	C_3H_6O
3	Formic acid	1.81	0.434	46	0.37	46	CH_2O_2
4	Butanal (syn.: Butyraldehyde)	1.97	0.472	189	0.34	72	C_4H_8O
5	3-Hydroxy-2 butanone	3.41	0.817	45	0.42	88	$C_4H_8O_2$
6	1,2-Propanediol	4.17	1.00	45	41.40	76	$C_3H_8O_2$
7	Furfural	6.14	1.472	96	0.67	96	$C_5H_4O_2$
8	*E*-3-Hexen-1-ol	6.70	1.606	41	0.17	100	$C_6H_{12}O$
9	*Z*-3-Hexen-1-ol	6.80	1.630	41	9.18	100	$C_6H_{12}O$
10	2,2,4-Trimethyl-1,3 dioxolane	10.40	2.494	43	2.02	116	$C_6H_{12}O_2$
11	*n*-Hexyl acetate	11.68	2.800	43	0.12	144	$C_8H_{16}O_2$
12	Limonene	12.16	2.916	68	0.22	136	$C_{10}H_{16}$
13	Linalool	14.44	3.462	71	0.17	154	$C_{10}H_{18}O$
14	*E*-Rose oxide	14.79	3.546	139	0.66	154	$C_{10}H_{18}O$
15	*α*-Terpineol (syn.: Menth-1-en-8-ol)	17.32	4.153	59	0.14	154	$C_{10}H_{18}O$
16	Nerol	18.4	4.412	69	5.62	154	$C_{10}H_{18}O$
17	Carvone	18.91	4.534	82	0.36	150	$C_{10}H_{14}O$
18	*γ*-Octalactone	19.31	4.630	85	2.98	142	$C_8H_{14}O_2$
19	Neryl acetate	22.22	5.328	69	0.17	196	$C_{12}H_{20}O_2$
20	*γ*-Decalactone	25.09	6.016	85	11.83	170	$C_{10}H_{18}O_2$
21	Neophytadiene	33.55	8.045	68	0.19	278	$C_{20}H_{38}$
Unidentified compounds 11.15%							
Identified compounds 88.85%		Oxygenated compounds 88.44%					
		Hydrocarbons compounds 00.41%					

*RT: Retention Time. **RRT: Relative Retention Time to 1,2-Propanediol.

Table 2: Identified compounds of PepFM from Headspace GC-MS.

No.	Name	RT*	RRT**	Base peak	Relative Area %	M. Weight	M. Formula
1	Acetone	1.73	0.102	43	0.19	58	C_3H_6O
2	Isobutanal	1.97	0.116	43	0.12	72	C_4H_8O
3	Acetic acid	2.18	0.128	43	0.14	60	$C_2H_4O_2$
4	Furfural	6.15	0.363	96	0.28	96	$C_5H_4O_2$
5	*α*-Pinene	9.13	0.539	93	1.41	136	$C_{10}H_{16}$
6	Camphene	9.60	0.567	93	0.10	136	$C_{10}H_{16}$
7	Methylcyclohexane	9.65	0.570	69	0.17	98	C_7H_{14}
8	*β*-Pinene	10.50	0.620	93	1.48	136	$C_{10}H_{16}$
9	Myrcene	10.97	0.648	41	0.45	136	$C_{10}H_{16}$
10	3-Octanol	11.40	0.674	59	1.22	130	$C_8H_{18}O$
11	*α*-Terpinene	11.78	0.696	121	0.08	136	$C_{10}H_{16}$
12	Limonene	12.19	0.720	68	5.48	163	$C_{10}H_{16}$
13	1,8-Cineole (syn.: Eucalyptol)	12.27	0.725	43	0.49	154	$C_{10}H_{18}O$
14	*γ*-Terpinene	13.15	0.777	93	0.15	136	$C_{10}H_{16}$
15	Linalool	14.40	0.851	71	0.18	154	$C_{10}H_{18}O$
16	Isopulegol	15.90	0.940	41	1.48	154	$C_{10}H_{18}O$
17	Menthan-3-one	16.27	0.962	112	26.11	154	$C_{10}H_{18}O$
18	Menthol	16.91	1.00	71	33.89	156	$C_{10}H_{20}O$
19	*α*-Terpineol (syn.: Menth-1-en-8-ol)	17.36	1.02	59	0.57	154	$C_{10}H_{18}O$
20	Pulegone	18.80	1.111	81	1.05	152	$C_{10}H_{16}O$
21	Carvone	18.93	1.119	82	0.11	150	$C_{10}H_{14}O$
22	Piperitone (syn.: Menth-1-en-3-one	19.24	1.137	82	1.03	152	$C_{10}H_{16}O$
23	Decanol	19.66	1.162	41	0.17	158	$C_{10}H_{22}O$
24	Menthyl acetate	19.79	1.170	95	0.07	198	$C_{12}H_{22}O_2$
25	*β*-Bourbonene	22.96	1.357	81	0.11	204	$C_{15}H_{24}$
26	*E*-Caryophyllene	23.91	1.141	41	0.31	204	$C_{15}H_{24}$
Unidentified compounds 23.16%							
Identified compounds 76.84%			Oxygenated compounds 67.10%				
			Hydrocarbons compounds 09.74%				

*RT: Retention Time. **RRT: Relative Retention Time to Menthol.

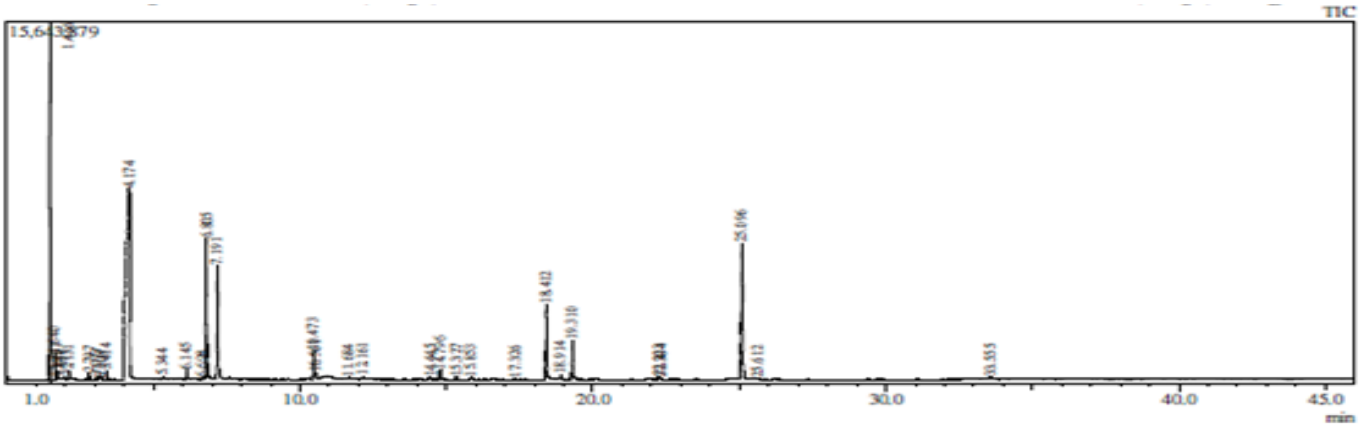

Figure 1: Total ion chromatograms (TIC) of MFM.

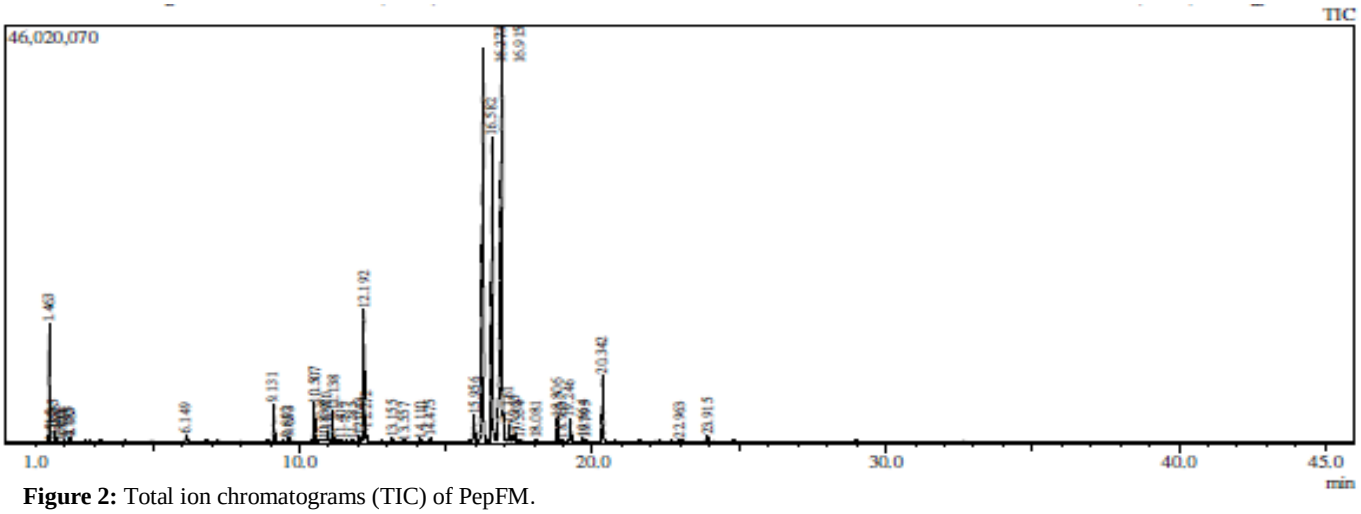

Figure 2: Total ion chromatograms (TIC) of PepFM.

The concentration of 1,2-propanediol increased the hazard of immune ailments and respiratory in children including hay fever, eczema, asthma and allergies from 50% to 180% [13,14].

While, the chief constituent was menthol (33.89%) in PepFM followed by menthan-3-one (26.11%). Menthol is the most commonly used in industry and the most tobacco additive in tobacco products marketed and advertised. Moreover, its diagnostic flavor. It has a variety of pharmacological effects enabling tobacco smoke inhalation and potentiating dependence. These characters of menthol not only favor tobacco initiation and consumption but also can prevent smoking cessation. Furthermore, it causes several chronic ailments and premature death [15].

Tobacco is one of the important cause of morbidity and mortality throughout the world. A recently infamous way of smoking tobacco is Waterpipe [16].

The smoke of a single cigarette contained many dangerous compounds such as 2.94 mg nicotine, 802 mg tar,145 mg CO, chrysene, phenanthrene and fluoranthene [17]. It is also a fact that the number of puffs and their volume from using A recently infamous way of smoking tobacco is Waterpipe are about 10 times higher than a cigarette and higher concentration of metals while the burning temperature for A recently infamous way of smoking tobacco is Waterpipe is about 900 °C as compared to 450 °C for a cigarette [18].

The peak concentration of nicotine in cigarette and Waterpipe are the same but the long duration of the Waterpipe use results in significantly greater effective nicotine exposure. Relative to a cigarette, Waterpipe smokers were exposed to 1.7 times the nicotine dose, when they were smoking tobacco through Waterpipe [19].

In this study, the anti-microbiological results indicated that the tested bacterial strains were susceptible to growth inhibition by MFM and PepFM methanol condensates. While, the tested fungus *C. albicans* showed also growth inhibition by the flavored Moâssel methanol condensates. These results are displayed in Tables (3-6).

In this study, we observed that a decrease in the microbial adherence achieved at higher concentrations of methanol condensates obtained after burning (above MIC). This is due to the growth inhibitory effects of methanol condensates, while at low concentrations (sub MIC), it was found that the microbial adherence increased. The results are demonstrated in Tables (7 and 8).

Tobacco smoke augments the binding of *Streptococcus pneumonia* to pulmonary epithelial cells by inducing eukaryotic platelet-activating factor receptor (PAF-R) expression, which interacts with phosphorylcholine on the cell wall of bacteria [20].

In *Porphyromonas gingivalis*, the major fimbrial protein, FimA, is unregulated, which aids adhesion by binding to the glyceraldehydes-3-phosphate dehydrogenase (GAPD, surface protein of the primary periodontal colonizer), *Streptococcus gordonii* reported that the principal nicotine metabolite and cotinine, increased *P. gingivalis* adhesion to epithelial cell monolayers [21-22].

It has become obvious that smoking promotes bacterial adhesion and biofilm formation in several other pathogens, including *Streptococcus mutans, S. aureus, P. aeruginosa* and *Streptococcus pneumoniae*. Enhanced bacterial development, including the emergence of antibiotic resistance, protection from antibiotics and other antimicrobials, immune response shielding and the increased potential for secondary colonization, each has clear implications to disease treatment for the current and the future [23].

Table 3: Inhibition zones (IZs) of different methanol condensates and standard antibiotics against the tested organisms in (mm).

Microorganism / Condensates & Antibiotics	*S. aureus*	*E. coli*	*K. pneumonia*	*P. aeruginosa*
MFM	8	7	8	10
PepFM	7	7	8	7
Ampicillin®	35	34	23	7
Clindamycin®	NA	NA	NA	NA
Gentamicin®	36	33	33	30
Amoxicillin/clavulnate (Augmenten®)	26	30	28	12
Unictam®	37	30	20	20

NA=Not active

Table 4: Minimum inhibitory concentrations (MICs) of different methanol condensates and standard antibiotics against the tested microorganisms (µg/mL).

Microorganism / Condensates & Antibiotics	*S. aureus*	*E. coli*	*K. pneumonia*	*P. aeruginosa*
MFM	1002	382.3	305.9	536.10
PepFM	1002	382.2	531.1	866.20
Ampicillin®	2.01	133.4	441.7	845.08
Clindamycin®	NA	NA	NA	NA
Gentamicin®	6.8	24.3	372.1	130.70
Amoxicillin/clavulnate (Augmenten®)	44.6	135.4	231.3	728.80
Unictam®	609.5	93.1	454.9	463.20

NA=Not active

Table 5: Inhibition zones (IZs) of different methanol condensates and Miconazole against *C. albicans* in (mm).

Antifungal & Condensates / Microorganism	Miconazole	MFM	PepFM
C. albicans	25	18	19

Table 6: Minimum inhibitory concentrations (MICs) of different methanol condensates and Miconazole against *C. albicans* fungus (µg/mL).

Antifungal &Condensates / Microorganism	Miconazole	MFM	PepFM
C. albicans	70.3	22.6	17.4

Table 7: The effect of MFM methanol condensate on microbial adherence.

Microorganism / Concentration	*S. aureus*	*E. coli*	*K. pneumonia*	*P. aeruginosa*	*C. albicans*
Control	0.212	0.253	0.262	0.331	0.235
Above MIC	0.189	0.107	0.166	0.249	0.168
	0.175	0.197	0.196	0.196	0.219
MIC	0.218	0.201	0.261	0.342	0.221
Sub MIC	0.268	0.289	0.290	0.408	0.475
	0.239	0.301	0.295	0.500	0.421

Table 8: The effect of PepFM methanol condensate on microbial adherence.

Microorganism / Concentration	*S. aureus*	*E. coli*	*K. pneumonia*	*P. aeruginosa*	*C. albicans*
Control	0.212	0.253	0.262	0.331	0.235
Above MIC	0.162	0.113	0.168	0.248	0.110
	0.165	0.179	0.178	0.272	0.210
MIC	0.201	0.221	0.251	0.326	0.229
Sub MIC	0.278	0.301	0.280	0.386	0.319
	0.289	0.301	0.220	0.389	0.337

Conclusion

There is a great difference in the chemical composition between the two flavored Moâssel products. However, the demonstrated antimicrobial activities of the tested methanol condensates, they are not recommended to use them as antimicrobial agents. Moâssel smoking is a very bad habit and may produce serious respiratory complications. So, do not use it at all.

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Conflict of interests

No potential conflict of interest was reported by the authors.

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