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Forensic chemical profiling of flavouring additives in seized mu'assel (shisha) by gas chromatography-mass spectrometry (GC-MS)

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Abstract

Background: Water-pipe tobacco smoking obsession has again spread worldwide. Especially, the younger generation is besotted with mu'assel (shisha) and avidly use these for smoking. Numbers of additives are being added in commercial brands of mu'assel (shisha) to impart diverse taste with amusing aroma. The name of only one of the masking flavours like strawberry, chocolate, vanilla etc. and not the chemical ingredients are printed on the packets of mu'assel. The manufactures remain silent about mentioning the chemical ingredients used for flavourings as they may not have scientific data about chemical compounds attributing to multiple flavours. There is also a shortfall in quality control owing to non-availability of technical procedure(s) to identify masking multiple flavouring additives. Many of these flavoured additives are either carcinogenic or potentially hazardous for human health. Ignoring health hazards, the avaricious manufacturers are intentionally adding multiple additives to make their products more addictive in order to increase their sales. The need of the hour is to unequivocally establish a technique for chemical profiling of the flavouring additives in mu'assel. In this paper, seven popular commercial brands of mu'assel were extracted, sonicated and analysed by GC-MS technique for detection of flavouring additives.

Results: Twenty-eight flavouring additives, i.e. camphor, linalool, benzyl ethanol, β-citronellol, menthol, vanillin, ethyl vanillin, eugenol, eucalptol, patchouli alcohol, nerol, rheosmin, musk ambrette, musk ketone, phenyl ethyl methyl ether, anethole, estragole, limonene, benzaldehyde, terpineol, phenyl ethyl butyrate, phenethyl isobutyrate, piperonal, methyl isobutyrate, methyl dihydro jasmonate, anisyl alcohol, trans-geraniol and sabinene along with nicotine were detected in varied proportions by GC-MS technique in seven seized popular commercial brands of mu'assel.

Conclusion: A study on chemical profiling of flavouring additives in commercial mu'assel has yet not been reported. Henceforth, this forensic attempt was aimed to secure public health by chemically profiling the flavouring additives of mu'assel. Many of the detected additives may cause severe health problems. Moreover, the smoker may suffer from neuroticism and psychoticism that may lead to a number of cases pertaining to physical assaults and sexual harassment.

Keywords: Forensic chemistry, Carcinogenic, Flavour, GC-MS, Nicotine, Smoking, Mu'assel, Hookah, Psychoticism, Public health

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Background

Tobacco is one of the most widely abused substances in the world and is found to be highly addictive for its major alkaloid stimulant—nicotine (Ralapati and Lu 1998). The tobacco plant is a part of the genus *Nicotiana* and of the *Solanaceae* (nightshade) family. While more than 70 species of tobacco are known, the chief commercial crop is *Nicotiana Tabacum* (Fig. 1).

Dried cured tobacco leaves are consumed by various ways such as in cigarettes, cigars, mu'assel, flavoured tobacco, snuff, chewing tobacco, dipping tobacco and snus. Among these, the most popular way to consume tobacco is mu'assel through a hookah or water pipe (Fig. 2) in which the vapour or smoke of mu'assel is passed through a water basin before inhalation (Wikipedia 2015). Mu'assel or shisha is becoming increasingly popular due to its flavoured tobacco smoke. It is a syrupy tobacco mix with molasses and vegetable glycerol as moisturiser. Some specific flavours are also added to it. Typical flavours of mu'assel include strawberry, chocolate, vanilla, apple, grape, guava, lemon and mint, as well as many other fruit-based mixtures. In this form, the user gets nicotine along with multiflavour smoke.

Additives are used primarily for flavours and smoothing the smoker's experience (Rabinoff et al. 2007). Ninety-five percent of chemicals used in fragrances are synthetic compounds derived from petroleum. Smoking them can cause cancer, birth defects, CNS disorders and allergic reactions, e.g. limonene—carcinogenic, benzyl alcohol—CNS depressants, camphor—CNS stimulant and linalool—CNS depressants (EPA 2015).

Some of these chemicals are also found addictive, e.g. menthol increases nicotine receptor density (Callier V 2014). Henceforth, the Tobacco Products Directive (2001/37/EC) was provided for EU member states to regulate tobacco products so as to protect public health. This directive included the prohibition of ingredients which increase the addictiveness of tobacco products (Europa 2015). Owing to the short falls in quality control norms and non-availability of a reliable facility to identify flavouring additives, avaricious manufacturers are intentionally adding additives without considering the adverse effect of these chemicals. Moreover, these manufacturers are only printing the name of the masking flavour and not the details of the other additives on the cover of the commercial mu'assel. Due to these unfair practices, smokers remain unaware about the health hazard of flavouring additives present in mu'assel. Some methods were developed for the detection of nicotine from tobacco leaves by GC-MS (Hossain AM et al. 2013) and for detection of furanic compounds in the smoke of the water pipe (Schubert et al. 2011).

Study on chemical profiling of flavouring additives in mu'assel has not yet been reported. Henceforth, a

Fig. 1 *Nicotiana Tabacum* (Wikipedia 2015)

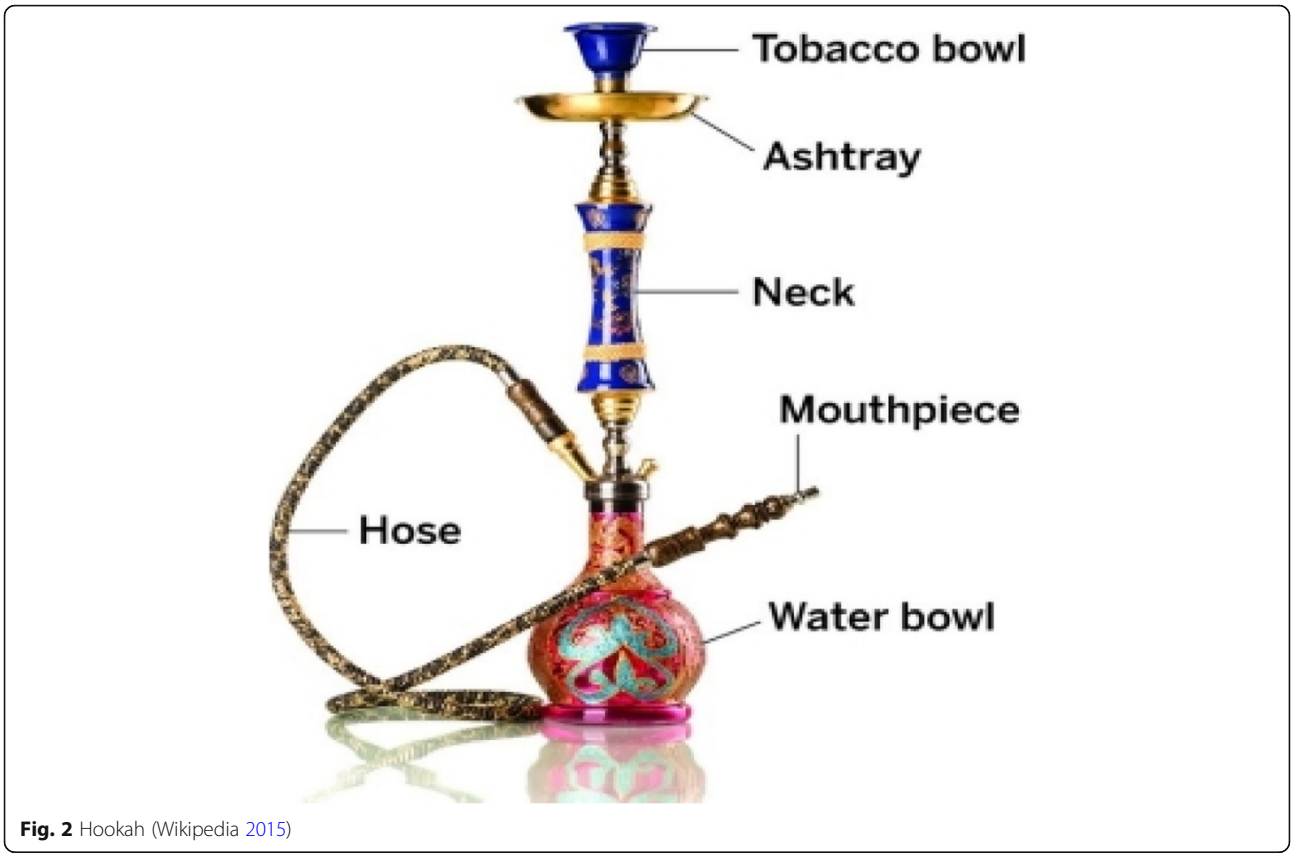


Fig. 2 Hookah (Wikipedia 2015)

forensic attempt is made to secure public health by chemically profiling flavouring additives in mu'assel. Forensic study was mainly targeted to give complete forensic chemical profiling of mu'assel so as to make smokers aware of the hidden health hazards of these products. In this study, representative samples from a large number of commercial varieties of mu'assel were subjected to forensic chemical analysis. These commercial varieties of mu'assel were seized during raids conducted in different hookah bars in Chandigarh by Chandigarh Police under COPTA Act, 2003, and were submitted at the Central Forensic Science Laboratory, Chandigarh, for the detection of nicotine. The samples of commercial varieties of mu'assel were ultrasonicated (Verma and Middha 2010) and analysed by using gas chromatography-mass spectrometry (GC-MS) technique. Twenty-eight flavouring additives apart from nicotine were identified by the mass spectral chemical profiling.

Materials and method

Representative varieties and reagents

The representative samples were taken from seven commercial varieties of mu'assel seized by Chandigarh police and were marked as R.V-1 to R.V-7.

Variety	Brand	Representative samples
1	Aladdin "Lady Dragon"	R.V-1
2	Aladdin "Sweet 16"	R.V-2
3	Aladdin "Brain Freezer"	R.V-3
4	Aladdin "X on Beach"	R.V-4
5	Qehwa "Pan Salsa"	R.V-5
6	Mya"Bombay Blue Pan "	R.V-6
7	Mya "Pan Rasna"	R.V-7

The packets of all the seized varieties of mu'assel were printed with only details of nicotine and tar, whereas the details of other multiple flavouring additives were not mentioned on any of the packets of mu'assel.

The solvent used for extraction was of LC grade (Merck, German).

Equipment

Ultrasonicator of PCi, Mumbai, India; Balance-AB104S of Mettler, Toledo, Switzerland; Thermo Finnigan Trace GC Ultra coupled with a Thermo DSQ Quadrupole MS and Thermo autosampler 3000 were used (directive 2001/37/EC).

Ultrasonic extractions of mu'assel for flavouring additives

Ten millilitres of chloroform was added to a 2-g representative sample of each variety in a round bottom flask and kept for 8 h in a refrigerator. These were then ultrasonicated for 1 followed by filtration through Whatman No.1 filter paper (GE Healthcare, UK). The filtrated samples were concentrated to 2 ml and stored at 4 °C.

Instrumentation conditions

A Thermo Finnigan Trace GC Ultra coupled with a Thermo DSQ Quadrupole MS and Thermo autosampler 3000 was used. The column was a 30-m BP-5 with 0.3-mm I.D. and 0.5-μm film thickness. Helium was used as a carrier gas at a constant flow of 1.2 ml/min. Splitless injection was used with a splitless time of 60 s. The injector and interface line temperature were held at 250 °C and 330 °C respectively. Oven temperature was held at 90 °C for 1 min and increased to 310 °C at the rate of 20 °CC/min and held at this temperature for 10 min.

The MSD conditions: ionisation energy 70 eV, ion source temperature 200 °C, mass range 41–410 amu, electron multiplier voltage (Auto tune + 200 V).

Sample injection volume: 1 μl

Compound identification

Xcaliber 1.4 software was used for data acquisition and processing and results were screened using the library of National Institute of Standard and Technology.

Results

Various chemicals apart from nicotine were detected in R.V-1 to R.V-7 by GC-MS technique. Out of these, 28 flavouring additives/ chemicals were found responsible for flavour in these representative varieties. These 28 flavouring chemicals are tabulated against their respective detected retention time (R_t) in Tables 1, 2, 3, 4, 5, 6 and 7 and their resulting total ion chromatogram are depicted in Figs. 3, 4, 5, 6, 7, 8 and 9 respectively. Combined chemical profiling of these 28 flavouring additives

Table 1 Detected flavouring additives in R.V-1

R_t	Chemical compound	Molecular weight (g/mol)	Important fragments
5.54	Limonene	136.24	68, 67, 93, 53, 94, 136
6.30	Linalool	154.25	71, 93, 55 ,121 ,136 ,139
6.51	Benzene ethanol	108.14	91, 92, 122, 65, 39, 123
6.91	camphor	152.24	95, 81, 69, 108, 152, 153
7.16	Menthol	156.27	71, 81, 95, 55, 123, 138
7.65	β-Citronellol	156.27	41, 69 ,81 ,82 ,123 ,138
7.93	Nerol	154.25	41, 69, 93, 123, 136, 154
8.31	Anethole	148.21	148, 147, 133, 77, 51, 149
8.96	Nicotine	162.2	84, 45, 133, 162, 55, 119
8.98	Eugenol	164.20	164, 149, 77, 55, 103, 165
9.40	Vanillin	152.15	151, 152, 81, 123, 53, 153
9.89	Ethyl Vanillin	166.18	137, 166, 109, 81, 29, 167
10.66	Rheosmin	164.20	107, 164, 43, 121, 94, 165
11.75	Patchouli alcohol	222.36	83, 138, 98, 222, 81, 55

Table 2 Detected flavouring additives in R.V-2

R_t	Chemical compound	Molecular weight (g/mol)	Important fragments
5.58	Eucalptol	154.249	81, 71, 108, 69, 111, 154
6.16	Phenyl ethyl methyl ether (Kewda ether)	136.19	45, 91, 136, 104, 65, 137
6.53	Benzeneethanol	108.14	91, 92, 65, 122, 39, 123
6.93	Camphor	152.24	95, 81, 69, 108, 41, 152
7.16	Menthol	156.27	71, 81, 95, 41, 123, 138
7.65	β-Citronellol	156.27	41, 69, 81, 82, 123, 138
7.98	Estragole	148.20	148, 147, 77, 121, 91, 51
8.33	Anethole	148.21	148, 147, 117, 77, 51, 149
8.84	Piperonal	150.13	149, 150, 63, 121, 65, 62
8.95	Nicotine	162.2	84, 133,162,42,119,65
8.98	Eugenol	164.20	164, 149, 77, 103, 55, 165
9.42	Vanillin	152.15	151, 152, 81, 123, 153, 154
9.89	Ethyl vanillin	166.18	137, 166, 109, 81, 29, 167
11.75	Patchouli alcohol	222.36	83, 138, 98, 222, 81, 55
12.91	Musk ambrette	268.26	253, 43, 91, 77, 268, 115

Table 3 Detected flavoured additives in R.V-3

R_t	Chemical compound	Molecular weight (g/mol)	Important fragments
5.54	Limonene	136.24	68, 67, 93, 94, 136, 137
5.86	Sabinene	136.23	93, 91, 71, 41, 136, 137
6.30	Linalool	154.25	71, 93, 43, 121, 136, 139
6.51	Benzene ethanol	108.14	91, 92, 65, 122, 39, 123
6.91	Camphor	152.24	95, 81, 108, 41, 152, 55
7.16	Menthol	156.27	71, 81, 95, 55, 123, 138
7.35	Terpineol	154.25	59, 93, 121, 136, 81, 139
7.65	β-Citronellol	156.27	41, 69, 81, 82, 123, 138
7.91	Trans-gerniol	154.25	69, 41, 68, 93, 123, 139
8.95	Nicotine	162.2	84, 133, 162, 42, 119, 65
8.98	Eugenol	164.20	164, 149, 77, 103, 55, 39
9.40	Vanillin	152.15	151, 152, 81, 123, 53, 153
9.72	Phenyl ethyl butyrate	192.25	104, 105, 71, 91, 106, 161
9.91	Ethyl vanillin	166.18	137, 138, 166, 109, 81, 167
10.66	Rheosmin	164.20	107, 43, 164, 77, 121, 165
12.91	Musk ambrette	268.26	253, 43, 91, 77, 268, 115
13.93	Musk ketone	294.30	43, 279, 128, 294, 115, 129

Table 4 Detected flavouring additives in R.V-4

R_t	Chemical compound	Molecular weight (g/mol)	Important fragments
5.60	Eucalptol	154.249	43, 81, 71, 108, 111, 154
5.81	Methyl isobutyrate	102.13	43, 40, 71, 87, 102, 103
6.16	Phenyl ethyl methyl ether	136.19	91, 136, 104, 65, 137, 138
6.30	Linalool	154.25	71, 43, 93, 55, 121, 136
6.51	Benzene ethanol	108.14	91, 92, 65, 122, 39, 123
6.93	Camphor	152.24	95, 81, 41, 108, 109, 152
7.16	Menthol	156.27	71, 81, 95, 55, 67, 123
7.65	β-Citronellol	156.27	41, 69, 81, 82, 123, 138
7.93	Nerol	154.25	41, 69, 68, 93, 123, 136
8.31	Anethole	148.21	148, 147, 117, 77, 105, 51
8.96	Nicotine	162.2	84, 133, 162, 92, 82, 163
8.98	Eugenol	164.20	164, 149, 77, 103, 55, 165
9.40	Vanillin	152.15	151, 152, 81, 123, 53, 153
9.74	Phenethyl isobutyrate	192.254	104, 43, 71, 105, 106, 42
10.66	Rheosmin	164.20	107, 43, 164, 77, 121, 65
11.45	Methyl dihydro jasmonate	226.32	83, 156, 153, 82, 55, 96
11.73	Patchouli alcohol	222.36	41, 43, 83, 98, 138, 222
12.89	Musk ambrette	268.2	253, 268, 91, 77, 115, 145
13.93	Musk ketone	294.30	43, 279, 128, 294, 115, 129

detected in R.V-1 to R.V-7 are summarised in Table 8 and the flavour of each detected chemical along with its chemical structure is summarised in Table 9.

Discussion

Twenty-eight flavouring additives were detected in seven representative varieties of mu'assel by GC-MS technique summarised in Tables 1, 2, 3, 4, 5, 6, ,7 and 8 and TICs of R.V-1 to R.V-7 are depicted in Figs. 3, 4, 5, 6, 7, 8 and 9 respectively. Some of these detected chemicals are derived from plants origin, some are synthesised and some are both: These are categorised as under:

Table 5 Detected flavouring additives in R.V-5

R_t	Chemical compound	Molecular weight (g/mol)	Important fragments
5.60	Eucalptol	154.249	81, 71, 108, 111, 154, 69
6.16	Phenyl ethyl methyl ether (Kewda ether)	136.19	91, 136, 45, 65, 104, 137
6.53	Benzene ethanol	108.14	91, 92, 65, 122, 64, 123
7.16	Menthol	156.27 l	71, 81, 95, 123, 138, 67
7.65	β-Citronellol	156.27	41, 69, 81, 82, 123, 138
7.93	Trans-gerniol	154.25	69, 41, 93, 123, 139, 154
8.96	Nicotine	162.2	84, 133, 162, 42, 119, 163
9.40	Vanillin	152.15	151, 152, 153, 81, 123, 53
11.75	Patchouli alcohol	222.36	83, 138, 98, 222, 81, 55
12.91	Musk ambrette	268.2	253, 43, 91, 268, 77, 115

Table 6 Detected flavouring additives in R.V-6

R_t	Chemical compound	Molecular weight (g/mol)	Important fragments
4.75	Benzaldehyde	106.12	105, 106, 77, 78, 107
5.60	Eucalptol	154.249	43, 81, 71, 108, 111, 154
6.17	Phenyl ethyl methyl ether	136.19	91, 136, 45, 65, 104, 103
6.30	Linalool	154.25	71, 93, 55, 69, 121, 136
6.53	Benzene ethanol	108.14	91, 92, 122, 65, 39, 93
6.93	Camphor	152.24	95, 81, 69, 108, 41, 152
7.16	Menthol	156.27	71, 81, 95, 82, 123, 138
7.65	β-Citronellol	156.27	41, 69, 81, 82, 123, 138
7.93	Trans-gernaniol	154.25	69, 41, 68, 93, 123, 139
8.28	Anisyl alcohol	138.17	138, 137, 109, 77, 94, 39
8.96	Nicotine	162.2	84, 133, 162, 42, 119, 163
8.98	Eugenol	164.20	164, 149, 77, 103, 131, 55
9.40	Vanillin	152.15	151, 152, 81, 109, 53, 50
9.89	Ethyl vanillin	166.18	137, 166, 138, 109, 81, 167
10.66	Rheosmin	164.20	107, 164, 43, 77, 121, 65
11.75	Patchouli alcohol	222.36	83, 138, 98, 222, 81, 55

Table 7 Detected flavouring additives in R.V-7

R_t	Chemical compound	Molecular weight (g/mol)	Important fragments
5.60	Eucalptol	154.249	43, 81, 108, 71, 111, 154
6.16	Phenylethylmethylether (Kewda ether)	136.19	45, 91, 136, 104, 65, 137
6.30	Linalool	154.25	71, 93, 55, 68, 121, 136
6.53	Benzene ethanol	108.14	91, 92, 122, 65, 39, 123
6.93	Camphor	152.24	95, 81, 69, 108, 41, 152
7.17	Menthol	156.27	71, 81, 95, 55, 123, 138
7.65	β-Citronellol	156.27	41, 69, 81, 82, 123, 138
7.91	Trans-geraniol	154.25	69, 41, 93, 123, 139, 154
8.31	Estragole	148.20	148, 147, 77, 121, 91, 51
8.95	Nicotine	162.2	84, 133, 162, 82, 92, 163
8.98	Eugenol	164.20	164, 149, 77, 103, 55, 165
9.40	Vanillin	152.15	151, 152, 81, 123, 53, 153
11.75	Patchouli alcohol	222.36	41, 43, 83, 98, 138, 222
12.91	Musk ambrette	268.26	253, 268, 43, 91, 77, 115
13.93	Musk Ketone	294.30	43, 279, 128, 294, 115, 129

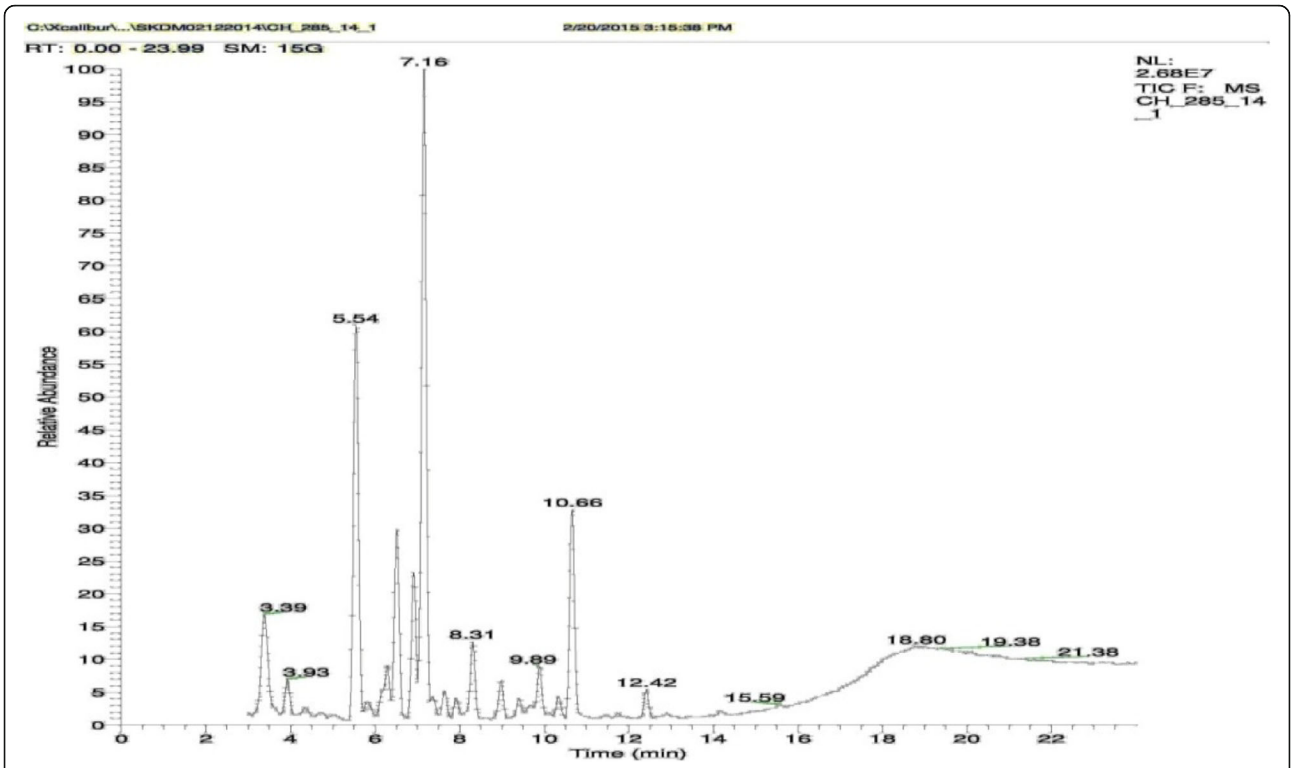


Fig. 3 Total ion chromatogram of R.V-1

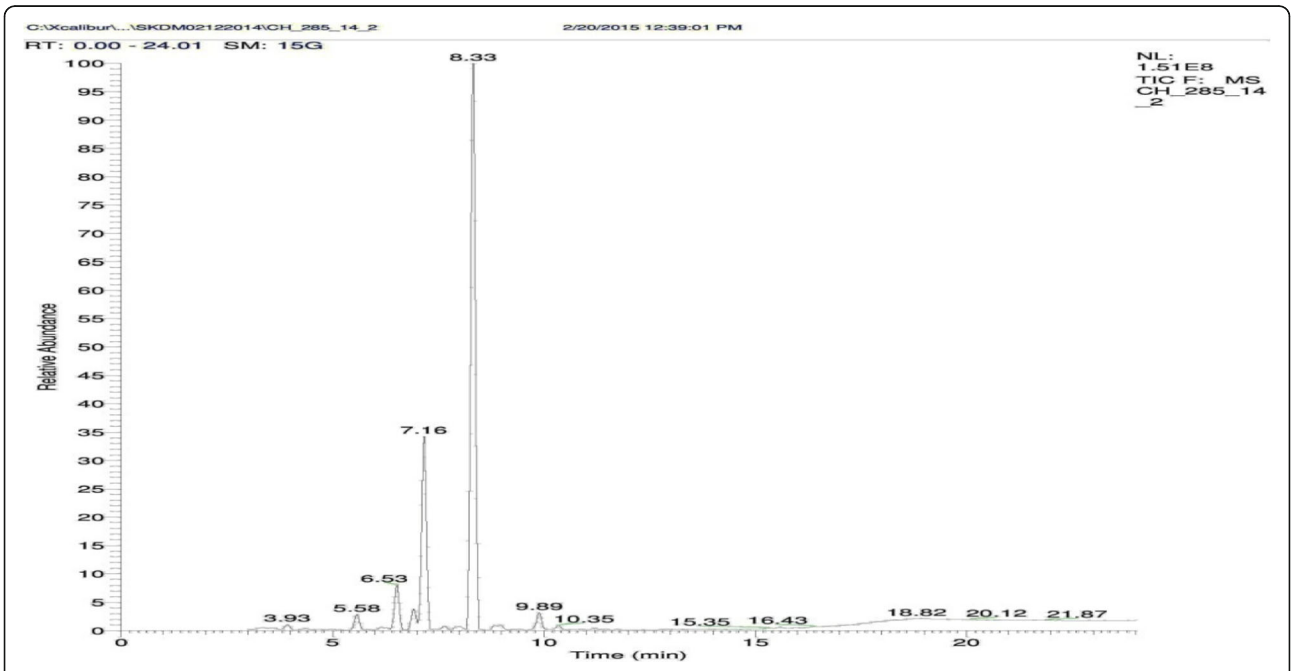


Fig. 4 Total ion chromatogram of R.V-2

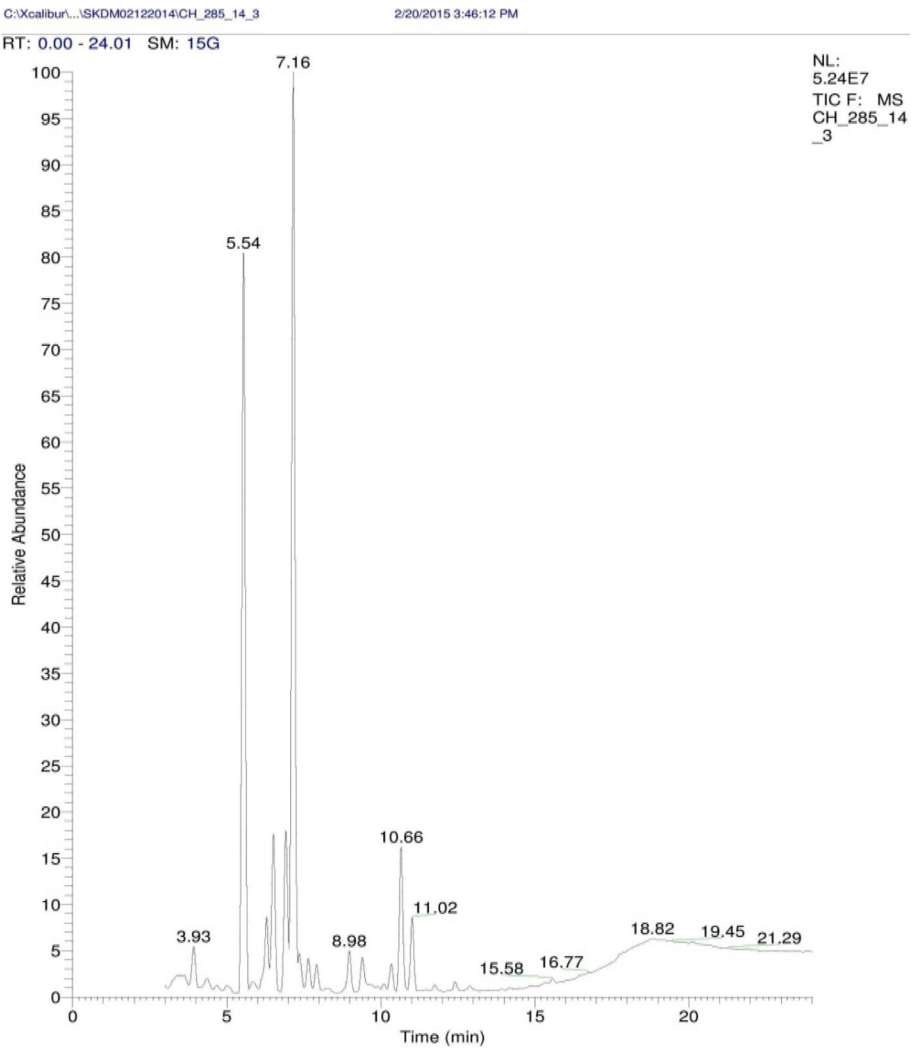


Fig. 5 Total ion chromatogram of R.V-3

1. Plant origin: camphor, linalool, benzyl alcohol, β-citronellol, menthol, vanillin, eugenol, patchouli alcohol, nerol, eucalptol, rheosmin, anethole, estragole, limonene, benzaldehyde, terpineol, trans-geraniol and sabinene
2. Synthetic: musk ambrette, musk ketone, ethyl vanillin, methyl isobutyrate, phenyl ethyl butyrate, phenethyl isobutyrate, phenyl ethyl methyl ether and methyl dihydro jasmonate
3. Both (plant origin as well as synthetic): piperonal and anisyl alcohol

These chemicals are added for imparting flavour into the mu'assel by the manufacturers. These detected chemicals owe specific properties for impartinga characteristic flavour. The flavour of each detected chemical is summarised in Table 9. Some of these chemicals impart similar flavours. The manufacturers inadvertently added on multiple chemicals imparting the same flavour in mu'assel, i.e for vanilla flavour—vanillin and ethyl vanillin in R.V-1, R.V-2, R.V-3 and R.V-6; for spicy flavour—eugenol and eucalptol in R.V-2, R.V-4, R.V-6 and R.V-7 and for musk aroma—musk ambrette and musk ketone in R.V-3, R.V-4 and R.V-7 and for aroma of mixture of citrus and floral—β-citronellol and methyl dihydro jasmonate in R.V-4.

TICs of seven representative varieties are depicted in Figs. 3, 4, 5, 6, 7, 8 and 9. Menthol was computed to have high proportion in R.V-1, R.V-3, R.V-5, R.V-6 and R.V-7, whereas anethole was in high proportion in R.V-2 and R.V-4. Patchouli alcohol, benzene ethanol and camphor were detected in R.V-1, R.V-4, R.V-6 and R.V-7; eucalptol in R.V-2 and R.V-4 to R.V-7; linalool in R.V-1, R.V-3, R.V-4, R.V-6 and R.V-7 and musk

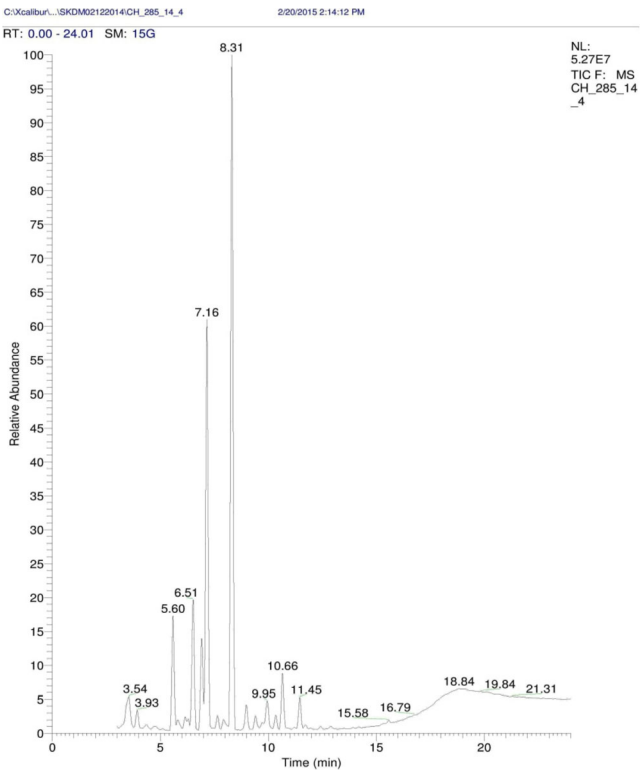


Fig. 6 Total ion chromatogram of R.V-4

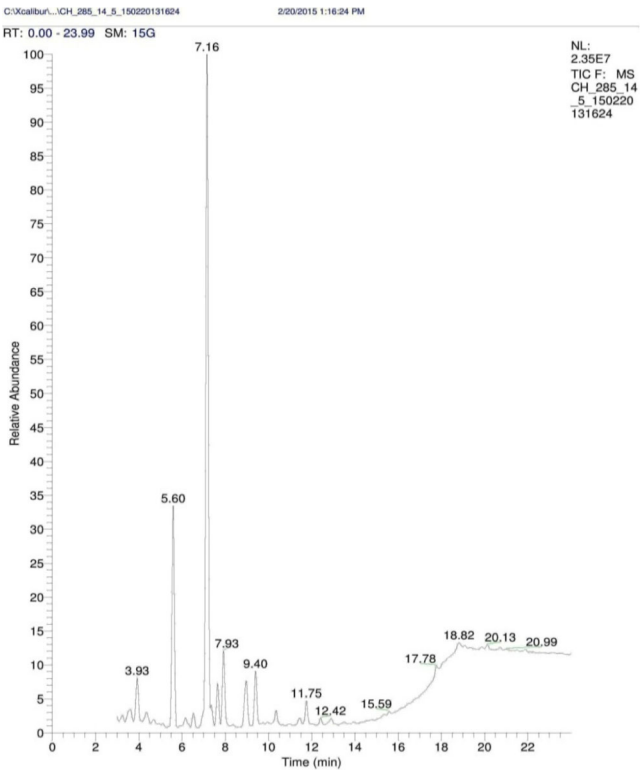


Fig. 7 Total ion chromatogram of R.V-5

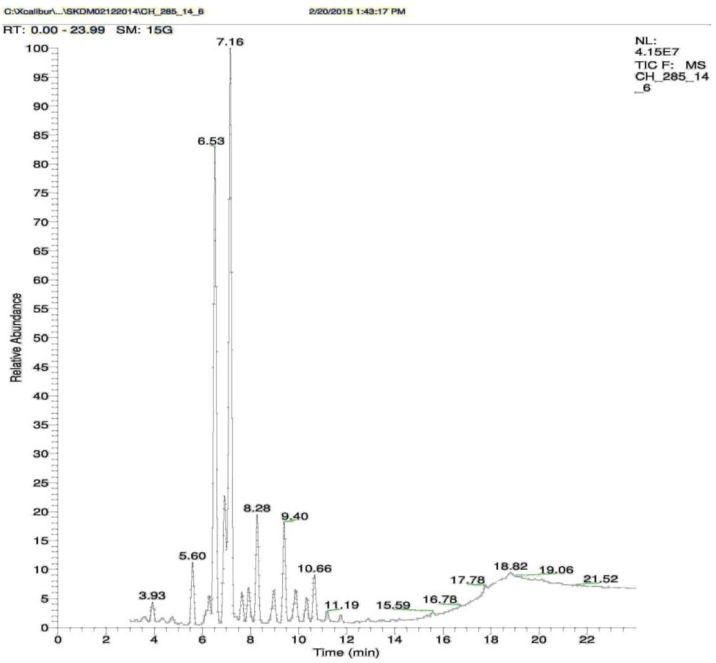


Fig. 8 Total ion chromatogram of R.V-6

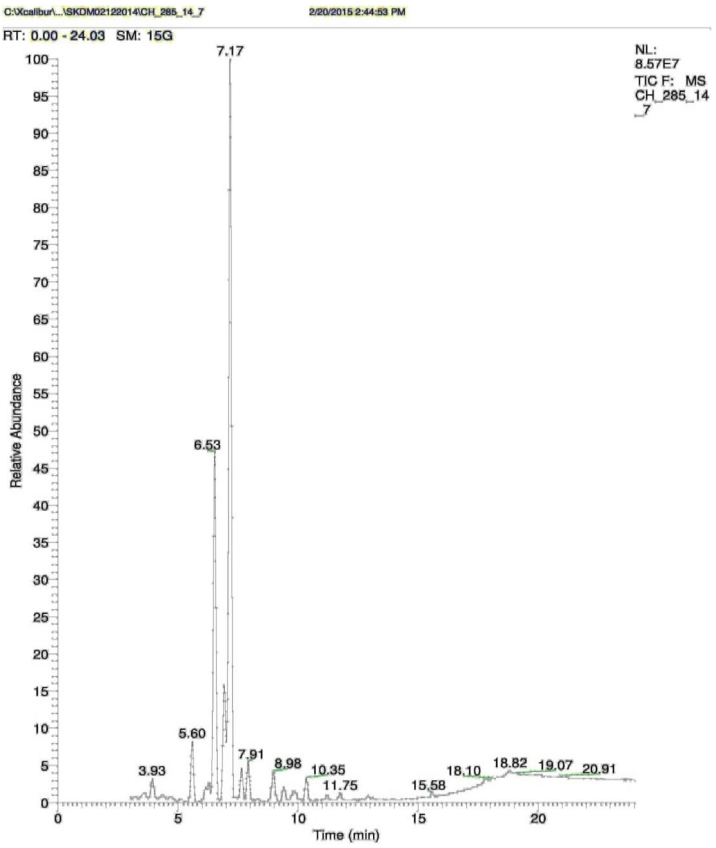
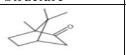
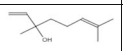
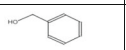
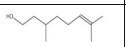
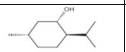
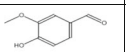
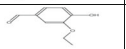
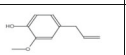
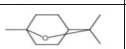
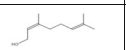
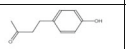
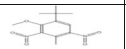
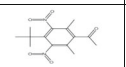
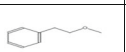
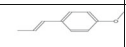
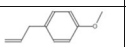
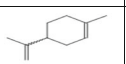
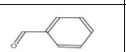
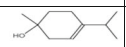
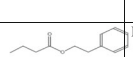
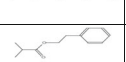
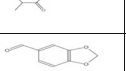
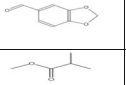
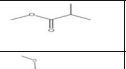
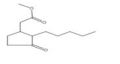
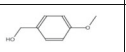
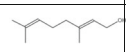


Fig. 9 Total ion chromatogram of R.V-7

Table 8 Combined chemical profiling of detected flavouring additives

S.No.	R.V-1	R.V-2	R.V-3	R.V-4	R.V-5	R.V-6	R.V-7
1	Camphor	Camphor	Camphor	Camphor	–	Camphor	Camphor
2	Linalool	–	Linalool	Linalool	–	Linalool	Linalool
3	Benzene ethanol	Benzene ethanol	Benzene ethanol	Benzene ethanol	Benzene ethanol	Benzene ethanol	Benzene ethanol
4	β-Citronellol	β-Citronellol	β-Citronellol	β-Citronellol	β-Citronellol	β-Citronellol	β-Citronellol
5	Menthol	Menthol	Menthol	Menthol	Menthol	Menthol	Menthol
6	Vanillin	Vanillin	Vanillin	Vanillin	Vanillin	Vanillin	Vanillin
7	Ethyl vanillin	Ethyl vanillin	Ethyl vanillin	–	–	Ethyl vanillin	–
8	Eugenol	Eugenol	Eugenol	Eugenol	–	Eugenol	Eugenol
9	–	Eucalptol	–	Eucalptol	Eucalptol	Eucalptol	Eucalptol
10	Patchouli alcohol	Patchouli alcohol	–	Patchouli alcohol	Patchouli alcohol	Patchouli alcohol	Patchouli alcohol
11	Nerol	–	–	Nerol	–	–	–
12	Rheosmin	–	Rheosmin	Rheosmin	–	Rheosmin	–
13	–	Musk ambrette	Musk ambrette	Musk ambrette	Musk ambrette	–	Musk ambrette
14	–	–	Musk ketone	Musk ketone	–	–	Musk ketone
15	–	Phenyl ethyl methyl ether (Kewda ether)	–	Phenyl ethyl methyl ether (Kewda ether)	Phenyl ethyl methyl ether (Kewda ether)	Phenyl ethyl methyl ether (Kewda ether)	Phenyl ethyl methyl ether (Kewda ether)
16	Anethole	Anethole	–	Anethole	–	–	–
17	–	Estragole	–	–	–	–	Estragole
18	Limonene	–	Limonene	–	–	–	–
19	–	–	–	–	–	Benzaldehyde	–
20	–	–	Terpineol	–	–	–	–
21	–	–	Phenyl ethyl butyrate	–	–	–	–
22	–	–	–	Phenethyl isobutyrate	–	–	–
23	–	Piperonal	–	–	–	–	–
24	–	–	–	Methyl iso Butyrate	–	–	
25	–	–	–	Methyl dihydro jasmonate	–	–	–
26	–	–	–	–	–	Anisyl alcohol	–
27	–	–	Trans-geraniol	–	Trans-geraniol	Trans-geraniol	Trans-geraniol
28	–	–	Sabinene	–	–	–	–

Table 9 Chemicals detected in R.V-1 to R.V-7 imparting the flavour

S.No.	Chemical compound	Structure	Flavour (aroma & taste)
1.	Camphor Formula:$C_{10}H_{16}O$ MW:152.24g/mol		Strong penetrating fragrant odour a bitter pungent taste
2.	Linalool Formula:$C_{10}H_{18}O$ MW:154.25g/mol		Pleasant scent (floral, with a touch of spiciness)
3.	Benzyl alcohol Formula:C_7H_80 MW:108.14g/mol		Mild pleasant aromatic odour
4.	β-Citronellol Formula:$C_{10}H_{20}O$ MW:156.27g/mol		Flora, citrus-rosy, fresh, fruity
5.	Menthol Formula:$C_{10}H_{20}O$ MW:156.27g/mol		Cool mint flavour
6.	Vanillin Formula:$C_8H_8O_3$ MW: 152.15g/mol		Vanilla taste and aroma
7.	Ethyl vanillin Formula:$C_9H_{10}O_3$ MW:166.18g/mol		Vanilla taste and aroma
8.	Eugenol Formula:$C_{10}H_{12}O_2$ MW:164.20g/mol		Spicy flavour
9.	Eucalptol (1,8-cineol, 1,8-cineole) Formula:$C_{10}H_{18}O$ MW: 154.249g/mol		Pleasant spicy aroma and taste
10.	Patchouli alcohol Formula:$C_{15}H_{26}O$ MW: 222.36		Patchouli scent
11.	Nerol Formula:$C_{10}H_{18}O$ MW: 154.25g/mol		Sweet rose odour like geraniol
12.	Rheosmin Formula:$C_{10}H_{12}O_2$ MW:164.20g/mol		Fruity odour of raspberry
13.	Musk ambrette Formula:$C_{12}H_{16}N_2O_5$ MW:268.26584g/mol		Musk type odour
14.	Musk ketone (white musk Formula:$C_{14}H_{18}N_2O_5$ MW:294.30312g/mol		Scent of deer musk or other natural musk
15.	Phenyl ethyl methyl ether (kewda) Formula:$C_9H_{12}0$ MW:136.19098g/mol		Green floral jasmine metallic fresh rose note
16.	Anethole Formula:$C_{10}H_{12}0$ MW:148.21g/mol		Odour and flavour of anise and fennel
17.	Estragole Formula:$C_{10}H_{12}O$ MW:148.20g/mol		Anisic type odour and an licorice type flavour
18.	Limonene Formula:$C_{10}H_{16}$ MW:136.24g/mol		Strong smell of oranges
19.	Benzaldehyde Formula:C_7H_6O MW:106.12g/mol		Almond like odour
20.	Terpineol Formula:$C_{10}H_{18}O$ MW:154.25g/mol		Pleasant odour similar to lilac
21.	Phenethyl butyrate Formula:$C_{12}H_{16}O_2$ MW: 192.25 g/mol		Floral odour
22.	Phenethyl isobutyrate Formula:$C_{12}H_{16}O_2$ MW:192.254 g/mol		Sweet Fruity- rose honey floral odour
23.	Piperonal (heliotropin) Formula:$C_8H_6O_3$ MW:150.13g/mol		Floral odour similar to that of vanillin or cherry
24.	Methyl iso butyrate Formula:$C_5H_{10}O_2$ MW:102.1317g/mol		Fruity odour of apple or pinapple
25.	Methyl dihydro jasmonate or Hedione Formula: $C_{13}H_{22}O_3$ MW: 226.32 g·mol−1		Mixture of Floral and citrus odour
26.	Anisyl alcohol Formula: $C_8H_{10}O_2$ MW: 138.17 g·mol−1		Odour like hawthorn
27.	Trans-Geraniol Formula: $C_{10}H_{18}O$ MW: 154.25 g·mol−1		Rose like floral flavour
28.	Sabinene Formula: $C_{10}H_{16}$ MW: 136.23 g/mol		Woody and Spiciness of black pepper flavour

ambrette in R.V-2 to R.V-5 and R.V-7. β-citronellol, menthol, vanillin and benzene ethanol were detected in all seven representative varieties of mu'assel.

The collective flavour of these chemicals in seven representative varieties of mu'assel are summarised in Table 10.

For imparting higher flavour notes, the avaricious manufacturers add multiple chemicals un-proportionally to mu'assel without considering their hazardous consequences. Moreover, to increase the sales of their products, manufacturers have been adding multiple chemical compounds having alike flavour. Owing to these, the fusion of flavours of these multiple chemical compounds in mu'assel does not have any specific pleasant flavour due to interference of flavours of various chemicals.

Conclusion

Twenty-eight chemicals responsible for flavour were detected in seven representative varieties of mu'assel. The presence of these flavoured additives varies in each of the seven representative varieties. A difference in the flavoured additives causes a difference in the flavour of these samples. These flavoured additives not only add flavour to these mu'assel products but also make the person more addictive for its taste. Moreover, buyers are not aware of these chemicals and their harmful effects as the details of these flavoured chemicals are not printed on covers of any of these representative varieties. Therefore, the manufacturer should print the name of all the added chemicals on covers of mu'assel packets.

The users while using hookah inhale nicotine along with these chemicals. Some of the chemicals along with nicotine are accumulated inthe human body while some are converted to new compounds during the heating process and also get accumulated in the human body. These chemicals and their new converted chemicals are harmful to the body. Some of these chemicals have been approved as additives to food but these are not tested by burning. The aforementioned health hazards need utmost attention by the public authorities; on our part, we have indeed carried out an in-depth analysis on the burnt flavouring additives of Mu'assel vis-à-vis health issues. The findings are being critically reviewed before making in public.

Forensic significance

Production and consumption of flavoured mu'assel are based on various economic, social, cultural and government policies. Due to loopholes in the government policy, they are commercially available in plenty in the market/online purchase and especially in hookah bars. The consumption of hookah along with flavoured additives is a major issue related to health as these are so hazardous that their exposure to the human body can cause inflammation to monocytes and can cause severe health problems. Some of these are neurotoxic and some are even carcinogenic. Another aspect of the flavoured mu'assel is the increasing mortalities due to the disease caused by smoking hookah and by consumption of flavoured additives.

The hookah smokers tend to be more extroverted, tensed, impulsive, depressive, anxious and suffered from traits of neuroticism and psychoticism which results in a number of cases pertaining to physical assault and sexual harassment. To make the situation worse, there is no internationally accepted standard

Table 10 Collective flavour (taste and aroma) by flavouring additives in mu'assel varieties

Representative sample	Collective taste	Collective aroma
R.V-1	Cool mint, vanilla, anise, fennel, citrus-rosy with spiciness	Strong penetrating odour of orange, anise, fennel, sweet rose, raspberry and patchouli
R.V-2	Cool mint, citrus-rosy, vanilla, licorice with spiciness	Strong penetrating odour of musk, green floral jasmine metallic fresh rose note, anise and fennel anisic type odour.
R.V-3	Cool mint, citrus-rosy, vanilla, woody with spiciness of black pepper	Strong penetrating odour of musk, vanilla, raspberry, orange, lilac and rose
R.V-4	Cool mint, citrus-rosy, vanilla with spiciness	Strong penetrating odour of patchouli, rose, spicy, raspberry, musk, green floral jasmine metallic fresh rose note, anise, fennel, fruity odour of apple or pineapple, sweet fruity-rose honey floral odour, mixture of floral and citrus
R.V-5	Cool mint, vanilla with spiciness	Citrus-rosy, patchouli, spicy, musk, green floral jasmine metallic fresh rose note
R.V-6	Cool mint, vanilla, spicy, rose-like floral	Strong penetrating odour of citrus-rosy, patchouli, spicy, vanilla, fruity odour of raspberry, green floral jasmine metallic fresh rose note and almond odour-like hawthorn
R.V-7	Floral, cool mint, vanilla, spicy, musk, licorice type and rose-like floral	Strong penetrating, floral with touch of spiciness, citrus-rosy, vanilla, spicy, patchouli, musk, green floral jasmine metallic fresh rose note and anisic-type odour

method for analysing flavoured mu'assel in forensic cases along with any type of database.

This research publication does have a societical outreach as it makes the public at large aware about the hazardous effects of flavouring additives in a mu'assel and will aid the public authority to make a smoke-free world.

Abbreviations

CNS: Central nervous system; COPTA: Cigarette and Other Tobacco Product Act; EC: European community; EU: European Union; GC-MS: Gas chromatography-mass spectrometry; LC: Liquid chromatography; MSD: Mass selective detector; R.V: Representative variety; TIC: Total ion chromatogram; WHO: World Health Organization

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Authors' contributions

Dr. DM has planned, reviewed, guided the research experimentations, interpretation and publication frame work. Mrs. AN has participated in methodology development, experimentations, interpretation and wrote the manuscript. Both authors have read and approved the final manuscript.

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Competing interests

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