

# An Analytical Study on the Common Type of Smokeless Tobacco Available in the Iranian Market

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**Background:** The use of smokeless tobacco is considered as a risk factor for oral cancer.

**Objectives:** The current study aimed to chemically analyze, separate and measure the existing substances in a type of chewing tobacco.

**Materials and Methods:** In the current descriptive study, the investigated sample was a type of smokeless tobacco, known as BT, manufactured in India. First steam distillation method and Clevenger machine were used to separate and extract the essential oil of the sample. The presence of the desired compounds was evaluated in the essential oil, using gas chromatography (GC) and then gas chromatography coupled to mass spectrometry (GC/MS) analysis.

**Results:** Based on the results obtained by the applied technique, the presence of carcinogenic compounds, N-nitrosomethyl-vinylamine (162 µg/g), N-nitrosornicotine (6.3 µg/g), and Acetaldehyde (117 µg/g) was confirmed in the sample.

**Conclusions:** Chemical analysis of the sample confirmed the existence of carcinogenic compounds.

**Keywords:** Analysis; Tobacco; Smokeless; Carcinogens

## 1. Background

Five million people lose their lives annually due to tobacco related diseases worldwide. According to the estimations, this rate rises to ten million in 2030 which exceeds the death rate caused by HIV, drug abuse, road accidents, murder, and suicide (1).

Tobacco is used in different forms including cigars, cigarettes, bidi, and smokeless tobacco (ST) that its compounds differ based on the region where it is consumed. Although people think smokeless tobacco is harmless, scientific evidence show that its consumption is a risk factor for blood pressure, dyslipidemia, abortion, low birth weight, exacerbation of asthma, and cancer (1, 2).

The smokeless tobacco contains large amounts of carcinogenic compounds among which Tobacco Specific N-nitrosamines (TSNAs) is the most important one (3).

4-methylnitrosamino-1-(3-pyridyl-1-butanone) (NNK) and N-nitrosornicotine (NNN) are the most important TSNAs existing in the smokeless tobacco. They exist in tobacco leaf in very low concentration and are produced in higher concentration during curing, aging, and tobacco fermentation phases due to the reaction between tobacco alkaloid amines and nitrite (4).

Oral tumors are developed in the laboratory animals using smokeless tobacco since 1981. Several investigators

showed the relationship between snuff consumption and oropharyngeal cancer in the laboratory animals (5). According to the researches by Hoffman et al. using NNN and NNK solutions resulted in tumors in mice and hamsters (5, 6). In another study, consumption of NNN caused different oral tumors in rats (7).

Rodu et al. after extensive study on the relative articles published from 1975, stated that the use of chewing tobacco and moist snuff are associated with low risk for oral cancer, while dry snuff is more risky (4).

Rickert et al. showed that the organic and aqueous extracts of smokeless tobacco are mutagenic or clastogenic (resulting in breakdown of chromosomes) (8).

Regarding the serious threats of smokeless tobacco consumption to human health, different studies were carried out to separate and measure its carcinogenic and toxic substances. Stepanov et al. investigated nitrosamines existing in 32 smokeless tobacco products in India's market. The highest level of nitrosamines was reported in different types of Zarda and Khaini (9).

According to Brunnemann's study, the level of NNK was from inseparable to 1.9 µg/g in the American and Danish samples, and the level of NNN ranged from 0.08 to 7.38 µg/g in the Danish and Belgian samples (10). In a study by

Hoffmann, about 30 carcinogenic substances were identified in chewing and snuff tobacco (5).

The present study was performed due to the following reasons: Sistan and Baluchistan is a province neighboring Afghanistan and India Pakistan through which these products are trafficked and presented in luxurious packages and distributed in the market; cultural and traditional similarities between the people living in the province, especially the Baluch, and those who live in neighboring countries; the relationship between consumption of smokeless tobacco and oral squamous cell carcinoma (11); and lack of study on identification of smokeless tobacco products, especially the most common type in the province's market.

## 2. Objectives

The current study aimed to analyze, separate, and measure the existing substances in a type of chewing tobacco.

## 3. Materials and Methods

### 3.1. Essential Oil Extraction

The studied sample was a type of smokeless tobacco known as BT and manufactured in India. To recognize the sample constituents, 25 g of it was placed in oven at 50°C to fully dry it out. Then, its essential oil was extracted using steam distillation and the Clevenger machine.

### 3.2. Identification of the Essential Oil Constituents

Gas chromatography (GC) and gas chromatography coupled to mass spectrometry (GC/MS) were used to identify the essential oil compounds. After injection of essential oil into GC apparatus, the essential oil constituents were qualitatively tested using time retention (TR), retention index (RI), and mass spectrum (MS), and these elements were compared with standard compounds or available information in the Saturn Library (12).

### 3.3. Calculation of Retention Index

To measure retention indices, normal C6-C30 alkane compounds were injected into GC apparatus. Using the information in the carcinogenic library and retention indices, the compounds mass spectra were identified and compared with the standard mass spectra. They were also compared with the retention indices published in different resources.

The compounds were quantified by means of data processor (R3 A-Chromatepac) using area normalization method and ignoring the spectra-related response factors.

### 3.4. Specifications of the Apparatuses

#### 3.4.1. Gas Chromatography

A gas chromatography apparatus (Shimadzu Company, GC-17A), equipped with a DB-1 type column (50 m length,

0.25 mm internal diameter, and 0.25 µm thin layer thickness) was employed for GC analysis. The injection site and detector sector temperatures were both at 225°C. The temperature was adjusted to start from 70°C and increase to 225°C with a ratio of 4°C/min. The carrier gas helium was applied with a speed of 1.1 mL/min.

#### 3.4.2. GC/MS Apparatus

The Shimadzu apparatus, QP 505, equipped with a DB-1 column (50 m length, 0.25 mm internal diameter, and 0.25 µm thin layer thickness) was used for GC/MS analysis. The electron ionization system with the energy of 70 eV was used for tracking. The oven temperature increased from 70°C to 225°C at a speed of 4°C/min. The carrier gas helium was applied with a speed of 1.1 mL/min.

## 4. Results

Based on the results obtained from the steam distillation and Clevenger machine, the existence of different compounds was confirmed. The results are presented in Table 1. By this method, carcinogenic compounds such as N-nitromethyl vinylamine, N-nitrosornicotine, and acetaldehyde were recognized.

**Table 1.** Chemical Compounds in the Studied Sample

Name	Percent	Retention Factor (I)
Hexane	18.83	600
1,2- Cineole	0.03	1139
L- Menthol	5.98	1094
Cyclohexanol	4.68	616
β-propiolactam	1.44	312
1-Dodeceny methyl	0.51	1300
Acetaldehyde <sup>a</sup>	2.05	324
N-Nitrosomethyl vinylamine <sup>a</sup>	2.83	344
Urotropinborane	2.32	2196
2,5-Divinyl-2-methyl-tetrahydrofuran	1.63	996
Isonomenthol	2.20	1026
Cis 3-hexenyl pyruvate	2.39	988
5-Octane-2-One, 6-Ethyl	1.85	978
2-Oxabicyclo [2.2.1] heptane	2.40	755
N-Allylaziridine	1.27	1098
2,3-Hexanedione	2.89	607
Butyric Acid	1.78	434
Propionaldimine	1.53	1342
Cyclohexanone	5.47	610
Citronellol	4.25	1023
2- Pentadecyn-1-O1	0.05	1514
Nonadecanol	0.05	1924
6-Ubdecanone	0.02	1138
Nicotine	0.62	1039
Eugenol	2.89	1046
N-Nitrosornicotine <sup>a</sup>	0.11	994

<sup>a</sup> carcinogen.

## 5. Discussion

Chemical analysis of the studied sample confirmed the existence of carcinogenic and toxic compounds. In different studies, the existence of volatile aldehydes, nitrosamines, nitrosamino acids, tobacco specific nitrosamines (TSNAs), and inorganic compounds such as carcinogenic substances are mentioned (5, 6, 11, 13). In the current studied sample, TSNAs carcinogenic compounds were also recognized. These compounds are made from precursors of alkaloids, nitrite, or nitrate during curing, aging, and fermentation of the tobacco. N-nitrosornicotine (NNN), N-nitrosoanabasine (NAB), and N-nitrosoanatabine (NAT) are created from the second-type amines of nornicotine, anatabine, and anabasin during the early stages of tobacco curing and fermentation, and NNK and some NNN are created from the third-type nicotineamines during the last stages of tobacco curing and fermentation (4, 5).

Several factors determine the concentration of TSNAs in the tobacco products such as the type of tobacco products, the amount of nitrate and nitrite, the curing technique, and the production methods. In addition, TSNAs have different levels in the various parts of the tobacco plant. Based on the analysis of 41 segments of tobacco leaf cured by air, the concentration of nitrosamines was smaller on the tip and the perimeter of tobacco leaf and was greater in the leaf base. In addition, TSNAs concentration was more dependent on the nitrogen and nitrite contents rather than the alkaloid content (14).

According to the above, due to differences in the product type, production feature, country of origin, and chemical analysis methods, it is difficult to compare the studied sample with the other samples. This is one of the study limitations, which is also confirmed by a broad range of reported TSNAs in the smokeless tobacco products. Smokeless tobacco accounts for more than one third of the tobacco products in India. The common forms of smokeless tobacco include tobacco with betel quid, lime, and tooth powder.

Based on the results of the analysis of these substances in India, the highest TSNAs levels were by Khaini and Zadra, and the lowest were reported in those of gutka and snuff. The amount of TSNAs in the betel and tobacco tooth powder could not be measured (5, 9).

In the sample under study, 6.3 µg/g of the NNN compound was identified. In a study by Stepanov et al. on new chewing tobacco products, the lowest amount of NNN was observed in different types of Ariva and Stone Wall (0.26-0.28 µg/g), and its highest level was reported in the Exalt (3.3 µg/g), which was lower than that of NNN level in BT (15).

Hoffmann et al. analyzed five samples of chewing tobacco in America and reported the highest amount of NNN in Copenhagen (8.73 ± 1.44 µg/g) and the lowest in that of Hawken (3.07 ± 0.3 µg/g) (6). Based on this study, the amount of NNN in Copenhagen is greater than in BT, and in Hawken is lower than in BT.

According to the study by Brunnemann, the level of NNN ranged from 0.08 to 7.38 µg/g in Danish samples and Belgian samples, which was comparable with that of the NNN existing in BT (6.3 µg/g) (10).

According to the study by Stepanov et al. on 32 products in India's market, the highest level of NNN was in Khaini and Zadra (0.08-28.4 µg/g), which was higher than that of the NNN level in BT (9). The concentration of NNN in BT sample was greater than those of some American, Danish, and Indian (Khiwam and Mishri) products, while it was lower than those of different types of Khaini and Zadra (Indian products) (9, 15).

Chemical analysis of a type of chewing tobacco, commercially known as BT and manufactured in India, using GC-MS apparatus proved the existence of carcinogenic and toxic substances in it. Therefore, informing people about disadvantages of such substances is essential.

Recommendations:

- 1) Analysis of chewing tobacco by thermal electrical analyzer (TEA), since it produces more powerful signals than GC-MS and is one of the methods to identify nitrosamine.
- 2) Separation of inorganic material by burning and examination of the sample using atomic absorption device.
- 3) Analysis of the other types of industrial and traditional chewing tobacco and their comparison.

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## Authors' Contributions

Dr. Mollashahi: study concept and supervision Dr. Noroozi: chemical analysis Dr. Afroughe: interpretation of data Dr. Hashemi: data collection.

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