Extraction and Determination of Nicotine in Tobacco from Selected Local Cigarettes Brands in Iraq

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Received: 11.01.2021; Revised: 10.05.2021; Accepted: 15.05.2021; Published: 27.06.2021

Abstract: Tobacco smoke contains more than 3,800 different compounds, where all could harm the exposed humans to different degrees. Among them, nicotine (3-(1-methyl-2-pyrrolidinyl) pyridine) is considered the most toxic compound. In many countries, tobacco smoking is considered a severe health hazard and a significant factor in death and several common diseases. Because of that, knowing the toxicity of nicotine is important to help understand tobacco-induced human diseases and identify the potential risks associated with the therapeutic use of nicotine as an aid in smoking cessation. Thereby, estimating the nicotine amount is very crucial in tobacco production. As this work aims to extract and determine nicotine, which is the highest toxic component in tobacco plant leaves, several methods were carried out to extract the nicotine. All extracting methods were based on water extraction as a polar solvent and the other non-polar solvents. Water shows a limited extraction activity for nicotine from tobacco leaves. Since nicotine is a dibasic compound, an alkaline solution (40% NaOH) was used to extract nicotine from tobacco leaves for the selected samples. Further, solvent extraction by non-polar solvents was conducted to extract nicotine from the alkaline solution and prepare it for the spectrometric analysis. FT-IR and UV analysis show that the oily substance, which was extracted from tobacco leaves, is nicotine. Results were confirmed by microscopic examination for the extracted nicotine after the addition of mercuric chloride, where flowery shape crystals of nicotine liganded with mercuric chloride complex were formed.

Keywords: nicotine; tobacco; solvent extraction; cigarette; smoking.

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1. Introduction

Tobacco smoke is a combination of around 5,000 chemicals with dynamic and reactive properties [1]. These components are generally toxic and carcinogenic, where they could exemplify a major source of toxic chemicals that affect humans’ health and initiate different diseases [2]. Based on the World Health Organization’s (WHO) data, smoking tobacco causes around 5.4 million early deaths globally [3]. However, as the trends increase, it is expected to have 10 million deaths yearly by 2025 because of tobacco smoking [4]. These deaths are mostly related to different types of cancer (particularly lung cancer), cardiovascular diseases, and inveterate respiratory diseases. Nevertheless, the surrounding of where smoking is consumed
is dangerous to other people, especially children, where various sicknesses could be developed just because of being close to smokers [5].

It was found that the number of individual chemicals that exist in the cigarette's smoke is unidentified but could exceed 100,000. Cigarette's pyrolysis could vary depending on the cigarette itself and/or the smoking-inhalation situation; hence the produced "chemicals' compositions are different [6]. The natural pyridine alkaloids, such as nicotine pyridine, naturally exist in the tobacco plant's leaves. Thereby, smoking cigarettes can be replaced with smokeless habits of tobacco products, for instance, chewing the tobacco itself or its gums, in order to avoid the smoke emissions [7].

The nicotine ratio in tobacco differs from one plant to another, depending on the tobacco type and planting conditions. Here, nicotine presents around 90% of the alkaloids that exist in tobacco, which equals nearly 1.5 wt.% nicotine (8.4 mg of nicotine for each cigarette) [8]. The other 10% of tobacco's alkaloids are nicotyrine, nornicotine, anatabine, anabasine, and myosmine [9].

The tobacco plant produces nicotine to protect itself from predators and insects since nicotine is toxic and can exterminate them. Nitrogen deficiency is a common problem in plants; therefore, nicotine is produced upon request to intensively take care of the product and keep its quality high [10]. The leaves of tobacco include more than 3800 recognized compounds, while there are 5000-6000 identified compounds during smoking. This indicates that there are nearly 2800 compounds produced from the smoking pyrolysis process that 'don't present in the plant [11].

When an individual inhales the cigarette's smoke, the nicotine condenses the dispersed particles in the lungs' fluid. This covers an area of 100 m² surface area of the 300 million pulmonary alveoli. Nicotine normally transfers from the blood to the brain at a rapid rate of 8–10 seconds without resistance and achieves the peripheral nervous system (see figure 1). This is even faster than the intravenous injection, putting the smoker into a trance and fulfill his/her desire by adjusting the nicotine levels in the blood. However, different factors are related to nicotine uptakes, such as the inhalation volume, depth, and frequency, in addition to the smoker's body conditions [11].

Figure 1. Nicotine concentration in blood plasma after smoking a cigarette (duration of smoking marked in blue).
Worldwide, 15 tons/day of nicotine from tobacco is roughly consumed by smokers [12]. That alone is sufficient reason for us to consider this particular natural product more closely. This work aims to extract and determine nicotine, which is the major toxic component in tobacco leaves.

2. Materials and Methods

Nicotine is a hydro carbonic component that contains nitrogen (alkaloid) in its structure. It is naturally existing in many plants, including tobacco, or can be synthesized. It is an oily liquid, hygroscopic, and miscible in water. The nicotine can result in solid-water soluble salts when mixed with acids. As a free base, nicotine burns in temperatures lower than its boiling point, while 35 °C is enough for its vapors' combustion in normal conditions. However, regarding cigarette smoking, the majority of the nicotine is combusted, and enough dosage is consumed to achieve the desired condition [13].

2.1. Sampling.

Two samples of common brands (Iraqi cigarette and Mu’assel) were purchased from the local market and compared to a raw tobacco sample. The raw tobacco was obtained from Sulaimaniyah/Iraq since that area is the major origin of tobacco in Iraq (see figure 2).

2.2. Materials.

Diethyl Ether (98%) (Fluka), Sodium Hydroxide (Sigma Aldrich), concentrated sulfuric acid 98% (Sigma Aldrich), Anhydrous Potassium Carbonate (BDH), Mercuric chloride (Sigma Aldrich).

2.3. Tobacco leaves extraction.

The extraction depends on isolating the base by dissolving the cigarettes in NaOH, then extracting nicotine from the filtrate by ether. After the evaporation of ether, nicotine oil can be obtained. The cigarette factories remove large quantities of nicotine from tobacco leaves because of their high toxicity. Thereby, the produced oil amount is very little [14].

Five grams of all samples were weighed and placed separately into a 250 ml beaker. About 150 ml of 40% NaOH solution was added. The contents were mixed by a magnetic stirrer thoroughly. Later, the flask with ingredients was placed on a hot plate in the fume
chamber and warmed to about 50 °C. The process was continued until a strong smell of tobacco started coming out of the flask. The Contents were cooled and filtered through Whatman 1 filter papers. The filter was washed with deionized water to ensure all the extracted solution was collected for the later step.

2.4. Nicotine extraction.

Nicotine is an oily yellowish-brown liquid with hygroscopic properties and easily dissolves with light petroleum, ether, and alcohol. Also, it is miscible with water in its base at a temperature range of 60-210 °C [15]. Several solvents were investigated as alkaloid extracting agents. This included isoctane, benzene, petroleum ether, and chloroform. Among the investigated hydrocarbons, Benzene showed higher satisfaction as an extracting solvent [16]. Hence, extracting the nicotine from tobacco leaves and purifying the nicotine could result in the best product. This is the reason for the extraction of nicotine by using a solvent extraction method. Later, in the extraction of nicotine as an alkaloid in tobacco, ether (could be petroleum ether) solvent is preferred to use because it is selective in dissolving the alkaloid substances. Using the right combination of ether and petroleum ether will optimize the time of extraction and nicotine production [17]. The extracted solution, obtained from section 2.3, was treated with 30 ml of diethyl ether using a separation funnel to extract the nicotine from the alkaline solution. This was repeated twice to ensure most of the nicotine was extracted by the solvent, as shown in figure 3 (a). Then, the two filtrates were gathered in a conical flask and dried using 1 teaspoon of anhydrous potassium carbonate, as shown in figure 3 (b), and then filtered. Finally, ether was evaporated in a water bath (Avoid extra heat because nicotine is hydrolyzed by extreme heating, considering that evaporation can be done at room temperature). The extracted nicotine was a yellowish oily liquid, as illustrated in figure 3 (c).

Figure 3. (a) Nicotine extraction by ether, (b) Drying by anhydrous potassium carbonate, and (c) Nicotine liquid.

Figure 4. (a) Nicotine extraction by kerosene and (b) Nicotine extraction by sulphuric acid.
As was mentioned earlier, nicotine is soluble in different types of solvents; this is the base for nicotine extraction from tobacco by using a solvent extraction method. The solvent choice is important because each solvent has its advantages and disadvantages [17]. As a non-expensive and safe solvent, 30 ml of kerosene was used to extract nicotine from the extracted solution of Iraqi tobacco obtained from sec 2.3. This process was repeated twice to ensure full nicotine extraction, as shown in figure 4 (a). The two filtrates were gathered in a separation funnel, as shown in figure 4 (b).

Nicotine was easily protonated by acid addition (figure 5) [18]. About 30 ml of concentrated sulfuric acid was added to the collected extract in a separation funnel.

![Figure 5. Mono and diprotonated nicotine.](https://nanobioletters.com/)

The dissolved nicotine in sulfuric acid was placed in a ceramic crucible and heated on a hotplate until the mixture reached its boiling point. Ultimately, the crucible was covered with a Petri dish to collect nicotine as water-soluble nicotinic salt.

### 3. Results and Discussion

Although all substances in tobacco smoke affect exposed humans to some degree, nicotine is generally considered as the main responsible substance for the pharmacological responses to smoking. In many countries, tobacco smoking is recognized as a serious health hazard and a major contributing factor to deaths from several common diseases. Consequently, awareness about nicotine's toxicity is important to know tobacco-induced human diseases, in addition to reaching the essential risks accompanied by the therapeutic use of nicotine to quit smoking [19].

#### 3.1. Nicotine extraction with an alkaline solution.

The chemical characteristics of nicotine are well known [8]. Pure nicotine is a colorless liquid with a characteristic acrimonious odor and boils at 246-247 °C. On exposure to air and light, or even placing in the dark in a sealed bottle, the colorless or pale-yellow oily liquid nicotine turns to brownish color over time. However, the brown color nicotine is as toxic as pure colorless or pale-yellow nicotine.

Nicotine could be charged and uncharged, so the pH largely affects its different physicochemical processes, like toxicity, excretion, absorption, and pharmacology. Nicotine is dibasic since its pyrrolidine and pyridine nitrogens pKa values at 15°C are 7.84 and 3.04, respectively. It was found that when pH= 7.4 and the temperature is 37°C, around 69% of the pyrrolidine's nitrogen ionize or gets a positive charge, while the pyridine's nitrogen is unionized [20]. Hence, the nicotine's solution pH significantly changes its protonated state. If the organic bases are charged, they have a hydrophilic nature; while if they don't, they are lipophilic. However, at a pH of 7.4, nicotine exists in two forms, so one can diffuse through the lipoprotein membranes, but the other can't. Like other alkaloids with close pKa values, Nicotine has a ratio...
of charged to uncharged forms of about 2 to 1 at a pH of 7.4. This chemical fact permits nicotine to have important biological actions.

3.1.1. Nicotine concentration.

Human exposure to nicotine increased in the last years due to the increasing forms of nicotine products in the market, which increased health issues. In addition to the previously available nicotine products, these products have different forms, such as smokeless tobacco and liquids for electronic nicotine delivery systems (electronic cigarettes). Unfortunately, most of these products are available worldwide without any consumption regulations. Based on previous researches and available information, a dose of 30–60 mg could kill the adult. Simply, 5 cigarettes containing 10 ml of the diluted nicotine solution probably lead to an adult death. The 60 mg dose could correspond to an oral median lethal dose (LD50) of 0.8 mg/kg, which is smaller than the determined values for animals tested in vitro (ranging from 3.3 for mice to higher than 50 mg/kg for rats) [21]. A typical cigarette contains approximately 0.5 to 1.0 g of tobacco and, on average, 10 mg of nicotine. A cigarette is typically smoked in 10 puffs and within 5 minutes. A typical smoker will absorb 1 to 2 mg of nicotine, but absorption can range from 0.5 to 3 mg [22, 23]. The elimination half-life of nicotine is 2 to 3 hours, meaning that the nicotine level in the blood decreases by one-half after a smoker stops smoking for that length of time [23].

The three samples used in this work were tested for nicotine concentration after alkaline extraction. Table 1 illustrates the calculated data for nicotine concentration.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Tobacco weight (g)</th>
<th>Nicotine weight (g)</th>
<th>Nicotine (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iraqi cigarette</td>
<td>5.00</td>
<td>0.08</td>
<td>1.6</td>
</tr>
<tr>
<td>Molasses</td>
<td>5.00</td>
<td>0.05</td>
<td>1.0</td>
</tr>
<tr>
<td>Iraqi raw tobacco</td>
<td>5.00</td>
<td>0.12</td>
<td>2.4</td>
</tr>
</tbody>
</table>

It is clear from table 1 that Iraqi tobacco contains more nicotine than Iraqi cigarettes. Both are higher than shisha tobacco (molasses) since it contains large amounts of flavors and other non-tobacco ingredients.

3.1.2. FT-IR analysis for nicotine.

FT-IR (ALPHA-Bruker) was used for instrumental analysis, the FT-IR spectrum of nicotine was illustrated in figure 6 [24].

![Figure 6. FT-IR spectrum for nicotine.](https://nanobioletters.com/)
From the figure, several peaks can be noticed, which characterize the different chemical functions of nicotine:

- Around 3400 cm\(^{-1}\), a large water peak can be seen (it deals with a liquid film).
- Between 2970 and 2780 cm\(^{-1}\): C-H stretching.
- The peak at 1677 cm\(^{-1}\): aromatic C=N double bond stretching.
- The peak at 1691 cm\(^{-1}\): aromatic C=C double bond stretching.
- The peaks at 717 cm\(^{-1}\) and 904 cm\(^{-1}\) correspond to the out-of-plane bending of the C-H bond of the mono-substituted pyridinic cycle.

FT-IR analysis was carried out for the extracted nicotine for one tobacco sample (Sumer Iraqi cigarette). Figures 7 shows the obtained FT-IR spectrum for the extracted nicotine from Sumer cigarette (the Iraqi cigarette) tobacco.

From figure 7, several peaks can be observed other than the original sample, which characterizes the different chemical functions of nicotine:

- Around 3337 cm\(^{-1}\), we can see the large peak of water.
- Between 2969 and 2780 cm\(^{-1}\): C-H stretching.
- The peak at 1585 cm\(^{-1}\): aromatic C=N double bond stretching.
- The peak at 1653 cm\(^{-1}\): aromatic C=C double bond stretching.
- The peaks at 712 cm\(^{-1}\) and 884 cm\(^{-1}\) correspond to the out-of-plane bending of the C-H bond of the monosubstituted pyridinic cycle.

To ensure that most of the nicotine in tobacco for the tested sample was extracted, the alkaline solution was tested after nicotine extraction by diethyl ether (figure 8 shows FT-IR analysis for the alkaline solution after nicotine extraction). FT-IR also tested the solid precipitate from filtration tobacco from alkaline solution to ensure that all nicotine was extracted (figure 9 shows the obtained spectrum for tobacco precipitate after nicotine extraction).
It is clear from figures 8 and 9 that the tested samples were freed from nicotine, and most of nicotine was extracted.

3.1.3. Ultraviolet analysis for nicotine.

The spectrophotometric analysis was conducted for nicotine in this research. However, nicotine in tobacco was preliminarily analyzed at the ATF National Laboratory Center using spectrophotometry. This test has many advantages, including rapid analysis, ease of use, capability, well establishment, and reliability. On the other hand, there are some disadvantages, where the main one is related to tobacco’s alkaloids structure. This means that the compounds’ UV absorption take place in the 260 nm region, which interferes with the absorbance of nicotine. Nevertheless, the ATF regulated tobacco products and considered all alkaloids as nicotine. It is important to ensure that the purity of nicotine is high to obtain the ideal UV absorbance measurements and provide valuable information about the standard nicotine chemistry. Nicotine is strongly absorbed in the UV at about 260 nm, as shown in figure 10, this absorbance depends on the pH of the used solvent. Figure 11 shows the UV spectra of nicotine in sodium phosphate buffer, pH 6.9 (A), deionized water (B), and sodium phosphate buffer, pH 2.5 [25].

Figure 9. FT-IR spectrum for tobacco precipitate after nicotine extraction.

Figure 10. UV absorbance spectrum of nicotine.
Figure 11. UV absorbance spectra of nicotine: (A) in 10 mM sodium phosphate buffer, pH 6.9, (B) in distilled, deionized water, and (C) in 25mM sodium phosphate buffer, pH 2.5.

UV analysis was carried out for the Iraqi cigarette tobacco sample's extracted nicotine using an Ultraviolet spectrometer (Shimadzu UV-1650PC). Figures 12 shows the obtained UV spectrum of the nicotine dissolved in diethyl ether at pH=5.5.

Figure 12. UV absorbance spectrum of nicotine for Iraqi cigarette tobacco.

UV absorbance for tobacco nicotine is absorbed in the 260 nm region. Alkaloids, such as nornicotine, are also absorbed in the 260 nm region, thus interfering with the absorbance of nicotine. Figure 12 shows the absorbance at 259.80 nm for the extracted nicotine in the presence of other alkaloids in the sample.

3.1.4. Microscopic examination for nicotine.

The heterocyclic nitrogen compounds are considered among the notable bio-ligands modules in bioinorganic and structural chemistry [26]. The widely studied type of these compounds is the six-membered heterocyclic, such as nitrogen pyridine, 2, 2’-bipyridine, and 2, 2’:6’, 2”-terpyridine. The five-membered ligands heterocyclic nitrogen compounds, such as pyrazoles and imidazoles, were also intensively utilized in inorganic chemistry [26, 27]. Only a few studies about the nicotine's organic and inorganic chemistry and its derivatives were reported by exploring the literature. At the same time, the majority report the preparation of new substituted nicotine derivatives and analogs due to their pharmacological applications as
potential chiral coordination ligands. Three crystal structures of metal complexes of nicotine had been previously reported. In these studies, the first crystal structure of a mercuric complex of nicotine was determined, describing a structure in which nicotine acts as a bridging ligand between the tetrahedral Hg(II) centers, forming a one-dimensional coordination polymer. The quantum-mechanical estimations aid in elucidating the experimental results as they give data on the interactions energy modes of Cu (II) with nicotine [28, 29].

Nicotine can be examined as a crystal shape after adding mercuric chloride to the nicotine extract with methanol, a flowery-shaped crystal formed [25]. Diethyl ether solution with the extracted nicotine from Sumer tobacco was examined to shape crystals after adding mercuric chloride. Figure 13 shows the obtained crystals shape examined under the microscope for the extracted nicotine. Nicotine acts as a bridging ligand between tetrahedral Hg (II) centers, forming a one-dimensional coordination polymer [30].

![Flowery crystal shape of nicotine bridging ligand with mercuric chloride](image1)

**Figure 13.** Flowery crystal shape of nicotine bridging ligand with mercuric chloride.

3.1.5. Nicotine extraction with kerosene.

Nicotine is a Diprotic Base with "'pKa’s of 3.12 (pyridine ring) and 8.02 (pyrrolidine ring) (see figure 14).

![Nicotine as a diprotic base](image2)

**Figure 14.** Nicotine as a diprotic base.

The nicotine molecule contains two nitrogen atoms capable of accepting a proton. The pyrrolidine nitrogen is considerably more basic than the pyridine nitrogen [31]. As a cheap and safe solvent, kerosene was used to extract nicotine from the extracted solution for Iraqi tobacco obtained from sec 2.3. Nicotine solution with kerosene dissolved in sulfuric acid, nicotine as water-soluble, nicotinic salt was obtained as dark brown drops.

3.2. **Nicotine extraction with deionized water.**

Nicotine is soluble in water and non-polar solvents. The same method of nicotine extraction was carried out using deionized water instead of the alkaline solution. Figure 15 shows the FT-IR spectrum for nicotine, clearly showing the absence of the major nicotine peaks.
in the obtained spectrum due to the slight nicotine solubility in neutral water compared with alkaline. Also, the wide peak of water around 3251 cm\(^{-1}\) may affect the other spectrum peaks.

![FT-IR spectrum for the extracted nicotine by water from Iraqi cigarette tobacco.](image)

**Figure 15.** FT-IR spectrum for the extracted nicotine by water from Iraqi cigarette tobacco.

### 4. Conclusions

Because of its toxicity and harmful effects on human lives, nicotine is the most important compound to be determined in tobacco. This work aimed to extract and determine nicotine, which is the major toxic component in tobacco leaves, using different methods. Tobacco cigarettes, tobacco shisha (called molasses), and raw tobacco were investigated and compared. All of the extracted methods were based on water extraction as a polar solvent and non-polar solvents. Water, as a neutral polar solvent, showed a limited extraction activity of nicotine from tobacco leaves. Because nicotine is a dibasic compound, an alkaline solution (40% NaOH) was used to extract nicotine from tobacco leaves for the selected samples. Further solvents extraction by non-polar solvents was carried out to extract nicotine from the alkaline solution and prepared for spectrometric analysis. FT-IR and UV analysis showed that the oily substance extracted from tobacco leaves is nicotine. Results were confirmed by the microscopic examination for the extracted nicotine after adding mercuric chloride to form flowery shape complex crystals of nicotine bridging ligand with mercuric chloride. Promising data was obtained when using ground tobacco leaves for water extraction, which may be recommended as a future work for nicotine extraction.

**Funding**

This research received no external funding.

**Acknowledgments**

The authors like to thank their affiliations for partially supporting this work.

**Conflicts of Interest**

The authors declare no conflict of interest.

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