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# Spectroscopic Study on *Tubacam Nicotina* Leaves and Commercial Tobacco in the Uv, Ir Regions And Its phytochemical screening of natural native tobacco from Libya

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**Abstract**
The leaves extracts of natural and commercial used tobacco sample tested on UV, IR spectrum and phytochemical screening. The colour of the natural tobacco extract was dark green, it's shape was mucilaginous and has pH 8.35, while the colour of the commercial tobacco extract was dark brown, it's shape was mucilaginous and has pH = 7.6. Furthermore, percentage yield of the raw extract of tobacco leaves plant and the raw extract of commercial tobacco were 12.723 and 7.341%, respectively. While percentage yield of alkaloids for tobacco leaves and alkaloids for commercial, tobacco was 6.32 and 4.13% correspondingly. The results of the qualitative detection tests revealed the presence of alkaloids in natural tobacco and commercial tobacco extracts. The UV spectrum of nicotine from *Tubacam nicotina* corresponds to the wavelength of the maximum absorption peak (λ Max of the compound) with the radiation spectrum of the standard compound also. Although the UV spectrum of nicotine form, commercial tobacco corresponds to the wavelength of the maximum absorption peak (λ Max of the compound) with the radiation spectrum of the standard compound. Furthermore, the absorption of nicotine in the infrared region, revealed to be identical to the IR absorption of the Nicotine standard, at 2917 cm where the vibration frequencies of the most critically active groups in the compound.

**Keywords:** *Tubacam nicotina*, Commercial Tobacco**,** UV, IR spectroscopy, Phytochemical Screening

## 1. Introduction
Tobacco is the common name for several plants of the *Nicotiana* genus and belongs to the Solanaceae (nightshade) family. Tobacco is a plant of about 2 meters tall with red flowers and large green leaves and owned about 60 different species. The most common plant species are tobacco smoke (*Tubacam nicotina*) [1, 2]. Nicotine is a stimulant and potent Para sympathomimetic alkaloid that is naturally produced in the nightshade family of plants, and is used as a treatment of addictions and ailments caused by tobacco misuse such as a smoking addiction where the substance can help relieve the recipient from withdrawal symptoms [3, 4, 5]. Likewise nicotine plays a very important rule in nicotinic acetylcholine receptors (nAChRs) [6, 7, 8], Correspondingly nicotine constitutes of 0.6–3.0% of the dry weight of tobacco [9], and usually compatible concentrations of nicotine varying from 2–7 µg/kg (20–70 millionths of a per cent wet weight), are found in the edible family Solanaceae such as potatoes, tomatoes, and eggplant [10]. Some studies indicate that the contribution of nicotine obtained from food is substantial in comparison to the inhalation of second-hand smoke [10]. Where others consider nicotine obtained from food to be trivial unless exceedingly high amounts of certain vegetables are eaten [10]. It functions as an anti-herbivore chemical; consequently, nicotine was widely used as an insecticide in the past [11, 12], and neonicotinoids, such as imidacloprid, are widely used. Nicotine is highly addictive [13, 14, 15] and one of the most commonly abused drugs [16]. An average cigarette yields about 2 mg of absorbed nicotine; high amounts can be more harmful [17]. Nicotine addiction involves drug-reinforced behavior, compulsive use, and relapse following abstinence [18]. Physical nicotine dependency involves tolerance, sensitization [19], physical dependence, and psychological dependency [20]. Nicotine dependency causes distress [21, 22]. Nicotine withdrawal symptoms include depressed mood, stress, anxiety, irritability, concentration difficulty, and sleep disturbances [23]. Mild nicotine withdrawal symptoms are measurable in excessive smokers, who experience normal moods only as their blood nicotine levels peak, with each cigarette [24]. On quitting, their withdrawal symptoms will worsen considerably, but they then will gradually improve to a normal state [24].

## 2. Materials and methods
### 2.1 Collection and preparation of plant material and Commercial Tobacco sample for extraction
**Preparing of the plant leaves of *Tubacam nicotina***
The plant leaves of *Tubacam nicotina* was collected from the Zeletin region, in Libya and identified at the Herbarium Section of the Department of Biology, College Of Sciences Al-Khums El-Mergib University Al-Khums Libya. The plant leaves were washed by tap water and then washed again with distilled water, and dried in the shade and the drying was finished in an oven at 45°C for 72 h. The dried leaves were

powdered by an electric grinder, then the powdered plant was size reduced with a sieve 200 Micron. The fine powder was then packed in an airtight container to avoid the effects of humidity and then stored at room temperature.

**2.2**. **Preparing of the Commercial Tobacco sample**

Two packets (20 cigarettes) of fine brand cigarettes of Commercial Tobacco purchased from the local market. Sample of the Commercial Tobacco dried in the shade for two days and finally drying was finished in the oven at 40°C for 72 h. The dried sample pulverized by an electric grinder, and then its size was reduced with a sieve 200 Micron.

**2.3**. **Extraction of the plant powdered leaves of *Tubacam nicotina***

20 g of the fine plant powder was boiled in 400 ml of isopropyl alcohol for 4 hours. Then, the mixture filtered off. The solvent in the filtrate was evaporated using a vacuum rotary evaporator at 45°C. The obtained dark green viscous extract was kept deeply frozen until further usage.

**2.4. Extraction of the Commercial Tobacco**

20 g of the fine powder of Commercial Tobacco sample was boiled in 400 ml of isopropyl alcohol for 4 hours. Then, the mixture was filtered. The solvent in the filtrate was evaporated using a vacuum rotary evaporator at 45°C. The obtained dark brown viscous extract was kept deeply frozen until further usage.

**3**. **PH measurement**

Inside a 100 mL flask, about 3g of the dry extracts were placed and then diluted with 30ml of distilled water. By using pH meter (HANNA Instruments) at 25 °C. Results showed in table 1.

**4**. **Phytochemical analysis**

The fine plant powder and the condensed extracts used for preliminary screening of secondary metabolites constituent such as alkaloid, tannins, saponins and flavonoids [25-26].

**4.1**. **Identification of alkaloids**

Each of the powdered of plant materials (1 g) were separately, boiled in a water bath with 10 ml of 5% Sulphuric acid in 50% ethanol. The mixture was cooled and filtered. With a portion set aside. Another portion of the filtrate was put in 50 ml of separating funnel and the solution made alkaline by adding 1-2 drops of concentrated ammonia solution. Equal volume of chloroform was added and shaken gently to allow the layers to separate. The lower chloroform layer ran off into a second separating funnel. The ammoniacal layer was reserved. The chloroform layer extracted with two quantities each of 5 ml of dilute Sulphuric acid. The various extracts then used for the following test:

**4.1.1. Mayer's test:** The filtrate was put in test tube I, 0.5 ml of Mayer's reagent was added drop by drop. Formation of a greenish colored or cream precipitate indicates the presence of alkaloids.

**4.1.2. Dragendorff's test:** The filtrate was put in test tube II, 0.5 ml of Dragendorff's reagent added drop by drop. Formation of a reddish-brown precipitate indicates the presence of alkaloids.

**4.1.3. Wagner's test:** The filtrate was put in test tube III, 0, 5 ml of Wagner's reagent added drop by drop. Formation of a reddish-brown precipitate indicates the presence of alkaloids.

**4.2. Identification of tannins**

Four g. of powdered plant material extracted by using 20 ml of 50% alcohol, it was then filtered and the filtrate was divided into three portions for the following tests:

**4.2.1. Bromine water test:** 4-5 drops of bromine water was added to the test tube I. A colored precipitate indicates condensed tannins while hydrolysable tannins gave none.

**4.2.2. Ferric chloride test:** 4-5 drops of diluted solution of $FeCl_3$ was added to the second portion of the filtrate; formation of a blue or greenish-black colour that changes to olive green as more ferric chloride added indicates the presence of tannins.

**4.2.3. Lead sub-acetate test:** 4-5 drops of lead sub-acetate solution added to the third portion. Occurrence of a colored precipitate indicates the presence of tannins.

**4.3. Identification of saponins**

**4.3.1. Frothing test: 1g** of the powdered plant material was placed in a test tube and 10 ml of distilled water was added and shaken vigorously for 1min. and then it was left standing still for 30 min and observed. Formation of honeycomb froth indicates the presence of saponins.

**4.4. Identification of flavonoids**

6 grams of the powdered plant material samples were completely de-tanned with acetone. The residue extracted with warm water after evaporating the acetone on a water bath. The mixture was then filtered while hot; the filtrate allowed to cool and used for the following test:

**4.4.1. FeCl3 test:** To test tube I, 2-3 drops of $FeCl_3$ solution added, production of greenish-black colour indicates the presence of phenolic nucleus.

**4.5. Identification of carbohydrates**

5 ml of crude extracts mixed with few drops of Molisch's reagent then 1 ml of concentrated $H_2SO_4$, Purple precipitate indicated the presence of Carbohydrates.

**5. Results and Discussion**

**Table 1:** Colour, shape and status of the raw extracts of *Tubacam nicotina* and commercial tobacco

| Plant name | pH | Colour of Extract | Shape and status |
|---|---|---|---|
| *Tubacam nicotina* | 7.6 | Dark Green | Mucilaginous |
| Commercial Tobacco | 7.4 | Dark brown | Mucilaginous |

Table 1 shows the physical properties of the raw extracts of *Tubacam nicotina* and commercial tobacco. The color of *Tubacam nicotina* was dark green, the shape and condition of it was wet gum, the = pH 7.6. The commercial tobacco color was dark brown, and the shape and condition were wet gum and the pH = 7.4.

**Table 2:** Results of Quantitive phytochemical analyses of the leaves *Tubacam nicotina* and commercial Tobacco extracts:

| Plant name | Percentages (%) | | | | |
|---|---|---|---|---|---|
| | **Yields** | **Alkaloids** | **Flavonoids** | **Tannins** | **Saponins** |
| *Tubacam nicotina* | 12.72 | 11.32 | 5.6 | 3.7 | 4.1 |
| Commercial Tobacco | 7.34 | 7.13 | 4.3 | 2.9 | 3.5 |

As shown in table 2 the percentage yields of each chemical constituent's present in *Tubacam nicotina* and Commercial Tobacco leaves were 12.72 and 7.34 % of extracts, while percentage yield of each alkaloid, Flavonoids, Tannins and Saponins in *Tubacam nicotina* were11.32- 5.3 3.7 and 4.1% correspondingly, and Commercial Tobacco7.13, 4.3, 2.9 and 3.5% respectively.

## Qualitative analysis

**Table 3:** Phytochemical screening results of crude extracts of leaves of the leaves *Tubacam nicotina* and Commercial Tobacco extracts:

| Chemical Constituent | Tests | *Tubacam nicotina* | Commercial Tobacco |
|---|---|---|---|
| Alkaloids | Mayer's | +++ | ++ |
| | Dragendorff's | +++ | ++ |
| | Wagner's | ++ | + |
| Tannins | Bromine water | ++ | ++ |
| | Ferric chloride | +++ | ++ |
| | Lead sub-acetate | + | + |
| Saponins | Frothing | ++ | + |
| | Hemolysis | - | - |
| Flavonoids | Ferric chloride | - | - |
| Carbohydrates | Molisch's | +++ | + |

The chemical ingredients are present as + = mild, ++ = moderate, +++= excess, - = absent.

Table 3 reveals the phytochemical screening of various chemical ingredients of selected plant species beneath study on a qualitative reason, were the phytochemical screening revealed the presence of most of the compounds such as Carbohydrates, Saponins, Alkaloids, and Tannins while Flavonoids not present in the crude extracts of leaves of *Tubacam nicotina* and Commercial Tobacco. Concentrations of phytochemical components in plants affected by environmental stresses such as drought, heat, cold, mineral deficiencies, plant diseases and others [27]. They also play key roles in helping plants adapt to their living environments [28]. The alkaloids concentration content increases in plants exposed to water stress [29], or increases under salinity stress conditions [30], and the metabolic response in the form of alkaloids known to many desert species [31].

## 6. Results of analysis of both local and commercial tobacco extracts using Ultra violet (UV) and Infrared (IR) Spectra

### 6.1. Results of analysis of Infrared (IR) Spectra

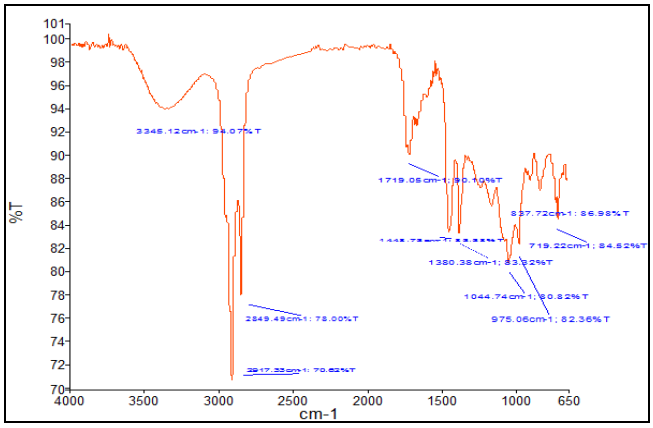


**Fig 1:** Results of analysis of Infrared (IR) Spectra for Commercial Tobacco

Infrared (IR) is one of the methods used in diagnosing of active organic compounds to measure their purity through beam sites and peaks. The infrared spectra is absorbed by nicotine, where the vibration frequencies of the most important and active groups in the compound were identical to the IR of the standard Nicotine, where at 2917 cm-1 a strong beam appeared at an implicit hydrogen bond between the hydroxyl and carbonyl groups. Moreover, a weak beam appears between 2917 - 3345. At 2849 – 2917 appears for the aromatic CH, additional beam appears between 1450 – 1719 which showed a strong and sharp carbon group C = H, and another beam appears between 1380 -1447 of medium sharpness C = C aromatic, Additional strong and sharp C-N beam emerged between 975 and 1044, one more beam at 1719 was strong , sharp and had a bending frequency for a group C = N as shown in Figure 1 [30-32]. On this spectrum, several peaks were observed, which are characterized by different chemical functions of nicotine: At about 3400 cm -1, the large top of the water molecule (dealing with a liquid film) can be seen. Also, observable between the range 2780 and 2970 cm -, while C-H stretching, similarly, a highest peak was observed at 1677 cm -1 which an expansion of aromatic bond C = N, Likewise, another peak appears at 1691 cm -1 which the extension of the aromatic bonds C = C. Whereas, the peaks at 717 cm -1 and 904 cm -1 correspond to the curvature outside the suspension or swinging of the C-bond of the single-replacement Pyridine rings.

### 6.2. Results of analysis of Ultra violet Spectra (UV)

#### 6.2.1. UV analysis of Nicotine extracted from commercial tobacco:

**Table 4:** Result of UV analysis of Nicotine extracted from commercial tobacco

| Abs | Wavelength (nm) |
|---|---|
| -0.004 | 730.0 |
| -0.002 | 695.0 |
| -0.005 | 585.0 |
| -0.005 | 375.0 |
| 0.000 | 355.0 |
| 0.013 | 330.0 |
| 0.061 | 300.0 |
| 0.046 | 290.0 |
| 0.239 | 275.0 |
| 10.000 | 255.0 |
| 10.000 | 235.0 |
| 10.000 | 210.0 |

As shown in table 4 the UV analysis results of the separated nicotine form commercial tobacco corresponds to the wavelength of the maximum absorption peak (λ Max of the compound) with the radiation spectrum of the standard compound, Which appeared at the wavelength = 235 nm and showed a high absorption value (λ Max and its value = 10 as shown in Table 4 and Figure 2 [32, 33, 34].

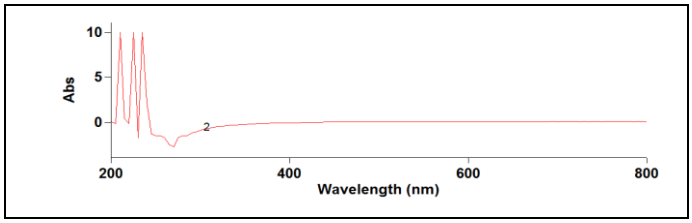


**Fig 2:** Nicotine compound extracted from commercial tobacco

#### 6.2.2. UV analysis of Nicotine extract from *Tubacam nicotina*

**Table 5:** Result of UV analysis of Nicotine extracted from *Tubacam nicotina*

| ***Tubacam nicotina*** | |
|---|---|
| **Peaks** | **Peak Style** |
| **0.0100** | **Peak Threshold** |
| 800.0nm to 200.0nm | Range |
| Abs | Wavelength (nm) |
| 0.075 | 730.0 |
| 0.044 | 594.0 |
| -1.543 | 280.0 |
| -1.576 | 255.0 |
| -1.417 | 245.0 |
| *10.000* | *239.0* |
| 0.717 | 223.0 |
| 0.361 | 217.0 |
| 0.072 | 214.0 |
| 0.218 | 212.0 |
| 0.204 | 210.0 |
| 0.801 | 208.0 |
| 0.418 | 204.0 |

The Nicotine (C10H14N2) is named as 3-(1-methyl-2-pyrrolidinyl) pyridine according to the IUPAC nomenclature whither mostly isolated from tobacco leaves. And as shown in table 5 the UV analysis results of Nicotine isolated from *Tubacam nicotina* corresponds to the wavelength of the maximum absorption peak (λ Max of the compound) with the radiation spectrum of the standard compound, Which appeared at wavelength = 239 nm and showed a high absorption value (λ Max and its value = 10 as shown also in Figure 3 [32, 33, 34]. Such as many previous studies have described, S-nicotine spectra to a minimum wavelength of approximately 210 nm and two variables set at 275 and 235 nm: a negative signal centered at 263 nm that shows a fine fibron structure is set to transition * π - π1 , While the positive range at 240 nm was set to * pyridyl n - π1 transition [35, 36]. Furthermore, in this research, two electronic transitions of nicotine between 200 and 239 nm described. The negative maximum of about 207 nm is either the n – s * or the charge transfer range resulting from the charging movement of the electron-free hydrogenation pair of N pyrrolidyl dependent N, to the * p orbit of the orbital pyridyl [37]. These observations summarize that nicotine and nor-nicotine enantiomers are

similar, but not identical, in their spectroscopic properties [37, 38, 39].

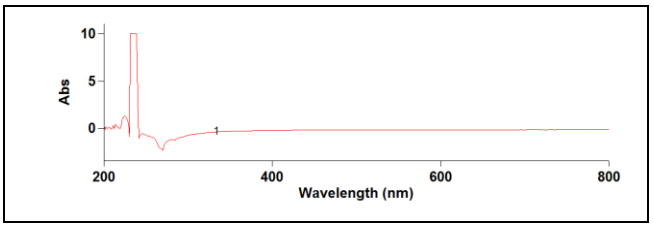


**Fig 3:** Nicotine compound extracted from *Tubacam nicotina*

## 7. Conclusions

This study was initiated to identify the medicinal importance of Tobacco, which is used as a traditional folk medicine by Libyans in ancient and current times. The results of the phytochemical tests revealed the presence of active chemical compounds additionally the UV and IR tests pointed to the existence of important chemical active groups, which also support the significance of tobacco as a medicinal plant.

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