

Quantitative Examination of Nicotine Content of Tobacco Leaves Using Spectrophotometric Method as a Means of Enlightenment Programme in Schools

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Abstract: This work is focused on the extraction and characterization of nicotine from tobacco leaves. The extraction of nicotine from tobacco leaf was done with solvent extraction method using methanol as the extraction solvent. The quantitative examination of nicotine content of the tobacco leaves was carried out using Ultraviolet-Visible spectrophotometer of a wavelength (λ_{max}) of 600nm. The results of the analysis revealed that the nicotine content of tobacco leaf is 1.564ppm. Nicotine can be significantly applied and used in industries, as insecticide because of its toxic property, it equally has various health problems on the organs and systems of people involved either directly or indirectly in the smoking of tobacco leaves on similar products. Such includes bronchitis, cyanosis, emphysema, cancer of the lungs, bladder cancer, cardiovascular diseases, cancer of the larynx and oesophagus especially to those who smoke tobacco leaves and tobacco products. In view of that, the researcher planned to carry out enlightenment campaign to different schools through different fora in order to educate the pupils, students, parents and teachers on the health problems associated with the use of tobacco through smoking and encourage them to disseminate the information to the public accordingly.

Key Words: Nicotine, tobacco leaves, methanol, Chemistry, students and enlightenment.

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I. Introduction

Tobacco has been in existence and smoked for several decades of years ago. It is available in different markets all over the world, even in the remotest part of the world, probably because, the use cuts across people of different age brackets; the children, adolescents and adults as the case maybe. Tobacco is a product prepared from the leaves of the tobacco plant by curing them (Wikipedia, n.d). The plant is categorized within the genus *Nicotiana* of the Solanaceae family (Jack, 1990). Tobacco was founded by Christopher Columbus in America in 1492 (Al-Darmon, Erhayem & Mohamed, 2015.) but ancient temple carvings showed that tobacco has existed and being smoked in Central America as long as 1000BC ago.

Tobacco contains the alkaloid nicotine, otherwise known as S-3-(1-methyl-2-pyrrolidiny) pyridine with a chemical formula, $C_{10}H_{14}N_2$. Nicotine is a stimulant and the most abundant of the volatile alkaloids in the tobacco leaf.

The primary commercial source of nicotine is by extraction from the plant *Nicotianatabacum* and *Nicotianarustica* (Al-Darmon et al., 2015). Nicotine is a colourless, light to pale yellow, hygroscopic oily liquid with unpleasant pungent odour, present in the leaf of *Nicotiana tabacum*, a nightshade solanaceae family of plants. It is synthesized in the root and predominantly accumulates in the leaves, making up 0.6 to 5% of the dry weight of tobacco. (Hadidi & Faisal, 2004; Byrd, Davis & Ogen, 2005). It is also found in lower concentration in tomatoes, eggplant, green pepper, red pepper, potatoes and also in the leaves of coca plant. (Hossain & Salehbuddin, 2013) Nicotine acts on nicotinic cholinergic receptors, affects most organ systems in the body and is highly addictive drug (Benowitz, 1996). It is one of the chemical components of tobacco leaves that has the ability of excited stimulation to the human system (Wei, Liu & Tang, 2018).

Dried tobacco leaves are mainly smoked in cigarette, cigars, pipe tobacco and flavored shisha tobacco and dipping tobacco. It has been a very worrisome experience after an encounter with the smokers in primary and secondary schools. No wonder the rate at which teenagers suffer from cancer, heart and kidney diseases has been on marginal increase. Some of these children start practicing act of smoking at home with wrapped papers. At this stage, concerned parents try to stop them early enough by keeping close eyes on such children. On the contrary, some other parents overlook the act and regard it as child play until the child becomes addictively interested in the act and gets into the real tobacco smoking at any slightest opportunity outside home: in the schools, and other public places including social gatherings.

According to Salawu, Damburam, Desalu, Olokoba & Agbo (2011), many children developed this habits as early as ten years. Some first timers learn from their parents, uncles, relations and friends. Habitual

tobacco users do that in order to reduce stress or for pleasure (Shu, Jung & Yu, 1994). Cigarette contains 8-20mg of nicotine (depending on the brand), but only approximately 1mg is actually absorbed in the human body (Benowitz, 1996). In lesser doses, nicotine acts as a stimulant in mammals, while high amount (50-100mg) can be harmful. This stimulant effect is the major contributing factor to the addictive properties of tobacco smoking.

In 2017, World Health Organization released a study on the environmental effects of tobacco. Most smokers begin much earlier and grow into the habit of making nicotine addiction difficult to control (Eriksen, et al, 2015). These younger smokers are at the greatest risk of future mortality (WHO, 2011). To buttress this point, World Health Organization, WHO, in 2015 revealed the death toll of tobacco smokers to be about seven million and by this year, it is certain that the number should have risen well above that.

Some experimental evidence have shown that nicotine is partly responsible for some of the life threatening diseases as chronic kidney disease, lung cancer and cardiac disease (Felman, 2018) in smokers. Center for Disease Control and Prevention (2013) in agreement, indicated that direct and indirect exposure to tobacco are the most important preventable causes of illness, disability and death among adults in the United State. Also implicated as the symptoms of prolonged exposure to nicotine according to (Asthana, et al. 2004) include, bronchitis, cyanosis, emphysema and disease of central Nervous system. Nicotine is associated with cardiovascular disease, potential birth defects and poisoning (Cosci et al., 2011). Tobacco use is a risk factor for many diseases, especially those affecting the heart, liver and lungs and several cancer in 2008, the World Health Organization (W.H.O., 2008). Excessive smoking has been implicated in lung cancer, bladder cancer and cancer of the larynx and oesophagus (Sunandra, Danda, & Dash, 2014).

Chemically, nicotine is a poisonous nitrogen containing chemical compound that has antimicrobial and insecticidal actions (Heydari, Mobidi, Mohammadi, Forouzandeh and Rashidzadeh, 2017). Medically, nicotine can be applied to rejuvenate the health conditions of schizophrenia patient (Goniewicz et al., 2017), dementia patient and skin mild cognitive dysfunction (Benowitz et al., 2018)

Since tobacco leaves contain variable concentrations of nicotine in different brands of tobacco, methods of extraction, variation in treatment as well as location, it becomes imminent to determine the concentration of nicotine content in order to enlighten as well as checkmate the adverse physiological effect of nicotine on peoples' health.

Sample collection and preparation.

Fresh tobacco leaves were purchased from Ogbete Main Market Enugu. They were washed thoroughly to remove dirt and sun dried for four (4) days. It was crushed into powder with mortar and pestle as well as electric blender, packaged in an air-tight container and stored in a refrigerator. All the glass wares used for this analysis were analytically prepared by first of all, washing with water and detergent, and further with, tetraoxochromate (vi) acid (H_2CrO_4) solution. They were finally rinsed with de-ionized water, dried in the oven and stored in a desiccator to avoid moisture until it is ready for use.

Standard Solution of Nicotine.

The solution was prepared by diluting the stock solution of Nicotine with methanol and deionized water. Eight solutions of nicotine were prepared by dilution of concentrated nicotine solution (1000mg/L) using concentration in the range of 0.21-1.62mg/L. All the chemicals used for nicotine extraction were analytically prepared, by proper standardization, in order to avoid being distorted by the interference effect of other chemical species in the nicotine standard curve.

Preparation of Standard Calibration Curve for Nicotine

The absorbance of each of the eight standard solution of Nicotine prepared, were individually determined using JENWAY 6305 UV-Visible spectrophotometer at a wavelength of 600nm against a reagent blank as reference. The instrument is ideal for this measurement because it provides the user with high quality and low cost effective measurement system for routine analysis. The absorbance values obtained were plotted against concentration to obtain the calibration curve.

Reagents/apparatus and extraction Procedure:

The reagents used include: 150ml beaker, 35ml double-deionized water, 1 ml of 2 molar solution of potassium hydroxide, 10ml of methanol, volumetric flask, zincethanoate solution, potassiumhexacyanoferrate (ii) solution, filter paper and centrifuge machine.

Extraction of nicotine.

This is the process of releasing nicotine from tobacco using an alkali and organic solvent. 1g of thoroughly washed and dried powdered tobacco leave was placed in a 150ml beaker and 10ml of methanol was added into the solution. The mixture was thoroughly shaken for 40 minutes at 200rpm. 35ml of deionized water

together with 1ml of 2M KOH were added into the mixture and stirred for another 40 minutes under the same condition.

The mixture was heated for 15mins to evaporate the methanol, allowed to cool and then filtered using a filter paper folded into a funnel. 1ml each of freshly prepared zinc ethanoate (CH_3COOZn) and potassium hexacyanoferrate (ii) ($\text{K}_4[\text{Fe}(\text{CN})_6]$) were added, before transferring same to 50ml volumetric flask and bringing up to the mark with double deionized water. The mixture was shaken and centrifuged for 4 minutes. The liquid portion was carefully decanted into a beaker and the residue was discarded. 0.5mg of fullers' earth was added to the decanted liquid. The mixture was shaken thoroughly and allowed to stand for 3 minutes at room temperature. The pH of the solution was raised by adding 0.01M solution of KOH to the mixture. The solution was filtered at this point and the final solution was made up to 50ml mark with deionized water. The total Nicotine content as well as the concentration were determined using the standard calibration curve.

II. Results

Table 1 contains the concentration and absorbance values of standard solution of nicotine.

S/N	Concentration(mg/L)	Absorbance (nm)
i.	0.213	0.102
ii.	0.401	0.391
iii.	0.624	0.603
iv.	0.802	0.782
v.	1.032	1.003
vi.	1.220	1.584
vii.	1.401	1.380
viii.	1.624	1.604

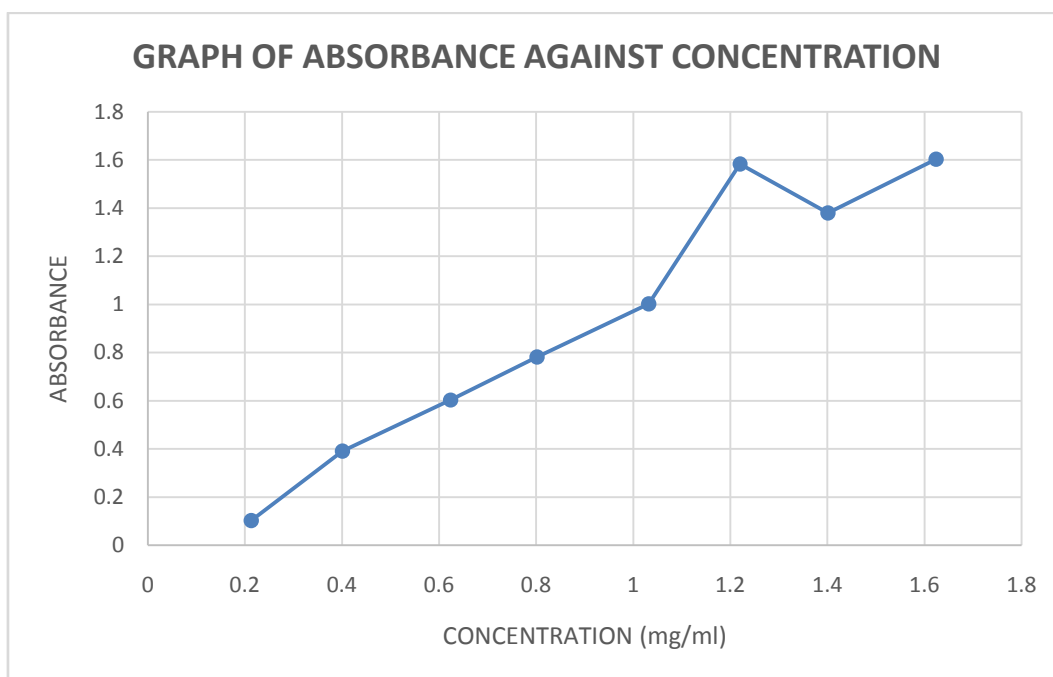


Table 2: Concentration of nicotine extracted from the leave of tobacco

Sample	Absorbance	Concentration mg/ml
Nicotine from Tobacco leaves	0.61	0.63

III. Discussion of Finding.

The range of the nicotine levels of tobacco leaves varies from location to location, depending upon factors like the leave position, and the variation in treatment involving, method of extraction and the nature of solvents used. The result of nicotine extracted from the leaves of tobacco as presented in table 2. Showed that

the concentration of nicotine in the tobacco leaf is 0.63. This indicates that the leaves of tobacco bought in Ogbete main market in Enugu, Enugu State, contains an appreciable concentration of nicotine ranging from (0.213-1.624). This value is within the range of the values of nicotine (0.7-3.26) recorded in the work of (Wang et al, 2008) carried out in China. Other works done in Ethiopian tobacco recorded higher nicotine values than this present work. These include 3.84 and 4.26, according to (Geto, et al 2012) and 2.04 and 2.54 as reported by (Kassa et al, 2013) in two other brands of the same Ethiopian tobacco.

IV. Conclusion

Tobacco leaves are rich sources of nicotine. Nicotine can be extracted from the leaves of tobacco using methanol as a solvent, the variation in the values of nicotine might be from the variation in the method of extraction, the reagents used, location or place of purchase and other environmental factors that may affect the potency of nicotine content of the tobacco in different countries. A host of other methods of extraction and characterization of nicotine from tobacco leaves are also available, among them are Column Chromatography method, High performance liquid chromatography with UV detection (HPLC-UV), Fourier-transform infrared spectrometric method (FT-IR), Thin-layer chromatographic method (TLC) and gas chromatography-mass spectrometry (GC-MS), Capillary electrophoresis, ASS, Circular dichroic spectrophotometry.

V. Recommendation

Researchers should try to carry out enlightenment campaign to schools by seeking audience from the school heads, to meet with the teachers, students and pupils in primary and secondary schools during their morning assembly to educate them on the health problems associated with the use of tobacco through smoking. They should also meet with the parents and teachers during PTA meetings in order to educate them on the dangers of life threatening diseases associated with tobacco smoking. Teachers and parents should be encouraged to propagate the information in their different classes while teaching, at home and other social gatherings. The frequent use of social media could be employed through whatsapp, facebook and instagram groups in order to disseminate the information to the public.

References

- [1]. Al-Darmon, M., Erhayem, M. & Mohamed, R. (2015). Analytical Determination of Nicotine Content in Tobacco Brands in Libyan Markets. **International Conference on Chemical, Civil and Environmental Engineering (CCEE)** Istanbul (Turkey) June, 5-6, pp. 49-53.
- [2]. Asthana, A. Rastogi, R.; Sunita, G.; & Gupta, V. K (2004). A Sample Spectrophotometric Method for the Determination of Nicotine in Environmental Samples. **Journal of the Chinese Chemical Society**, **51(5A)** 949-953.
- [3]. Benowitz, P. (1996). "The Cigarette Century the Rise, Fall and Deadly Persistence of the Product". **Nature** **16 (2)**, 134 – 146.
- [4]. Benowitz, N. L., Pipe, A., West, R., Hays, J. T., Tonstad, S., McRae, T & Anttenelli, R. M. (2018). Cardiovascular effects with smokeless tobacco use: comparison with cigarettes and nicotine Patch in Smokers: A Randomized Clinical Trial. **JAMA internal medicine**, **178(5)**: 622-631.
- [5]. Byrd, G. D.; Davis, R. A. & Ogden, M. W.(2005). A rapid LC-MS-MS Method for the determination of Nicotine and Cotinine in Serum and Saliva samples from smokers: Validation and comparison with a Radioimmunoassay Method. **Journal of Chromatographic Science**, **43(3)**, 133-140.
- [6]. Centers for Disease Control and Prevention (2013) Notes from the Field: Electron Cigarette use among middle and high school students., United States, 2011-2012. **MMWR, Morbidity and Mortality Weekly Report**. **62(35)** 729-730.
- [7]. Cosci, F., Pistelli, F., Lazzarini, B. & Carrozzi, L. (2011). "Nicotine Dependence and Psychological Distress, Outcomes and Clinical implications in smoking Cassation". **Psychology Research and Behaviour Management** **4**, 119-128.
- [8]. Eriksen, M. Machay, J. Schluger, N.; Islami, F. & Drope. J. (2015) **The tobacco atlas**, 5th ed. Atlanta Georgia: American cancer society.
- [9]. Felman, A. (2018). Everything you need to know about nicotine. Available, online from: <http://www.medicalnewstoday.com/articles/240820.php>.
- [10]. Geto, A.; Amare, M.; Tessema, M. & Admassie, S (2012). Poly(4-amino-3-hydroxynaphthalenesulfonic acid)-modified glassy carbon electrode. **Electroanalysis**. **24**: 659-665. Doi: 10.1002/elan.201100653.
- [11]. Goniewicz, M. L, Gawron, M., Smith, D. M., Peng M., Jacob, P. & Benowitz, N. L. (2017). Exposure to nicotine and selected toxicants in cigarette smokers who switched to electronic cigarettes a longitudinal within-subjects observational study. **Nicotine and Tobacco Research**; **19(2)**: 160-167.
- [12]. Hadidi, A.; Faisal, I. M. (2004). Nicotine content in Tobacco used in hubble-bubble smoking. **Saudi medical Journal**, **25 (7)**, 912-917.
- [13]. Heydari, R., Mobidi, A. M., Mohammadi, R., Forouzandeh, Z., & Rashidzadeh, S (2017). Effect of emotional distress and academic stress on level of nicotine addiction among medical students. **Nova Journal of Biological Sciences**. **6(1)**
- [14]. Hossain, A. M. & Salehuddin, S. M. (2013). Analytical determination of nicotine in tobacco leaves by gas chromatography-mass spectrometry. **Arabian Journal of Chemistry**, **6(3)**: 275-278.
- [15]. Jack, J. G. (1990). Tobacco: A Study of its Consumption in the United States John Wily Press London, 107-117.
- [16]. Kassa, H. Geto, A. & Admassie, S (2013). Voltametric determination of nicotine in cigarette tobacco at electrochemically activated glassy carbon electrode. **Bulletin of the chemical society of Ethiopia**, **27**: 321-328
- [17]. Salawu, F. K.; Damburam, A.; Desalu, A. R, Olokoba, A. B. & Agbo, J.(2011). Cigarette Smoking habits among adolescents in Northeast Nigeria. **Nigeria Postgraduate Medical Journal**. **18**: 26-29.
- [18]. Shu, Q. W., Jung, J. E. & Yu B. (1994). Cigarette Smoking and its risk factor among Senior High school students in Beijing China. **Tobacco control**, **3**: 107-104

- [19]. Sunandra, P., Danda, B., Dash, C. (2014). Prevalence of abnormal spermatozoa in tobacco chewing sub fertile males. *Journal of Human Reproductive Science*. 7(2): 136-142.
- [20]. Wang, L. Xiong, H. Zhang, X. Wang, S (2008). Electrochemical behaviors of Jain, & Jaimes (2013).nicotine and its interaction with DNA. **Electrochemistry Communication**. 11(11), 2129-2132. Doi: 10.1016/j.elecom.2009.09.012.
- [21]. Wei, X. N.; Liu, Y. & Tang, Y. L.(2018). Nicotine Content of tobacco leaf estimated by UV Spectrum. 4th international conference on Agricultural and Biological Sciences. **Earth and Environmental Sciences**, 185 (2018) 012017. 1-6.
- [22]. Wikipedia- Tobacco. Retrieved from. <http://en.wikipedia.org/>
- [23]. World Health Organization (2008). "Framework Convention on Tobacco Control WHO FCTC". W.H.O. Int. Retrieved. 09-18.
- [24]. World Health Organization (2011). Youth and Health risks. WHA64.28.http://who.int/hac/events/wha_a64_r28_en_youth_and_health_risks.pdf
- [25]. World Health Organization (2015). WHO Report on the Global Tobacco Epidemic, 2015. [online] http://apps.who.int/iris/bitstream/10665/178574/1/9789240694606_eng.pdf?ua=1&ua=1 assessed on: may, 16, 2019.
- [26]. World Health Organization (2017). Tobacco and its environmental impact: An overview. Online: <https://www.who.int>envi...8>

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