RESEARCH ARTICLE

Determination of level of nicotine in some commercial cigarettes available in Ethiopia using UV-Vis spectrometer

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ABSTRACT

Nicotine a bioactive alkaloid molecule which is a psychoactive chemical found in in cigarette. Level of nicotine in different cigarette brand are depends on different factor. Nicotine reacts with alkaline potassium permanganate to form a product having maximum absorbance (λ_{max}) at 610 nm. This observation made use of in a UV-vis spectroscopic determination of level of nicotine content in some commercial The content of variety of cigarettes brands studied were found to be as follows: Marlboro=16.4 ± 1.25 mg/g, Nyala premium=12.27 ± 1.46 mg/g, Rothmans=11.27 ± 1.35 mg/g, Gisila=11.0 ± 1.56 mg/g, Ghamdane=9.67 ± 1.30 mg/g, Nyalla=8.93 ± 0.35 mg/g, Royal=8.89 ± 0.25 mg/g, Delight=8.47 ± 0.45 mg/g, finally Kent cigarette were found to have lowest nicotine level which is 6.73 ± 0.87 mg/g. the result of this research also confirmed that the nicotine content of Ethiopian cigarette brands (13.19 mg/cigarettes) was a little bit smaller than level of nicotine in imported brands (15.30 mg/cigarette). However, level of nicotine in Ethiopian cigarette brands (10.17 ± 16 mg/g) have almost equivalent nicotine level when expressed in milligram per gram with imported cigarette brands (10. 59 ± 3.49 mg/g).

Key Words: *spectrophotometer, nicotine, potassium permanganate, cigarette, tobacco*

INTRODUCTION

The Cigarettes are smaller cylindrical roll of shredded tobacco leaves wrapped in cylinder of thin paper for smoking. Cigarettes is ignited at one end and allowed to smolders: its smoke is inheld from the other end, which is held in or to the mouth and in some cases a cigarette holder may be used as well. Usually, cigarette is made up of tobacco plant and tobacco plant naturally rich in variety of chemical components such as alkaloids [1]. In addition to the chemical components found in tobacco leaves, as much as six hundred different additives are used in cigarette manufacturing during fabrication [2]. Most modern manufactured cigarettes are filtered, filed with reconstituted tobacco and other additive chemicals which serve to enhance the sensory properties of cigarettes and the bioavailability of nicotine. Nicotine is the primary psychoactive chemicals in tobacco and there for makes cigarette to have addictive behaviors [3]. A key ingredient that makes cigarettes more addictive is the inclusion of reconstituted tobacco, which has additive to make nicotine more volatile as the cigarette burns. The amount and nature of additives vary from tobacco to tobacco and from factory to factory. Sometimes cigarette contains only one type of tobacco blend, but mostly it contains a mixture of tobacco blends [4]. level of nicotine in tobacco is the major index for evaluating the quality of tobacco and hence it is the best indication to show the quality of cigarettes. Level of nicotine in tobacco is affected by different factors [5]. Some of the factors which affects level of nicotine in tobacco are:

Tobacco type

the ability to synthesize nicotine or related alkaloid is a heritable peculiarity of the genus Nicotiana. Within the genus, certain species, notably N. Tabacum (tobacco) produce mostly nicotine as its alkaloids. Others such as N. Sylvestris contains closely similar alkaloid nornicotine and N. glauca contains anabasine as an alkaloid. Levels of alkaloid in tobacco plant also genetically controlled: N. rustica often contains much more nicotine as flue-cured varieties of N. tabacum [6]. Experimental studies indicate that among different

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tobacco oriental tobacco contains very low amount of nicotine; Virginia tobacco contains medium amount of nicotine and burley tobacco type contain comparatively large amount of nicotine.

Soil type

soil type on which a tobacco is cultivated is also another factor that affects level of nicotine in the tobacco plant. The quality of tobacco is directly related to the level of nicotine in the tobacco plants. Good quality of tobacco requires fertile, well-drained and moist soil. Soil can affect leaf size, texture, and color of tobacco. Sandy soil tend to produce relatively large leaf that is light in color and body, fine in texture and burn with a weak aroma. Heavier soil which contains silt and clay tend to produce a small, dark with a heavy body and strong aroma when burned. More over the amount of root development in the soil also affects the total nicotine contain of tobacco plant. That is when the depth of soil increases tobacco roots grow well and nicotine uptake from the root to the leaf becomes enhanced and large nicotine can be accommodated in the tobacco leaf. On the other hand, damage of roots by any agent minimizes nicotine content of tobacco plant.

Nitrogen nutrition

nitrogen nutrition is another factor which affect the nicotine level of tobacco plants. Nitrogen is directly consumed by tobacco plants from the soil as ammonium and nitrate compounds and nicotine contains 17.3% by mass of nitrogen in its molecular formula ($C_{10}H_{14}N_2$). the amount of nitrogen supplied to the soil has direct relationship with amount of nicotine in the tobacco plant. When tobacco plants are crowded together in dense stands, the leaves of tobacco plants do not attain full expansion and they becomes crowded for moisture and plant nutrients, nitrogen in particular can be expected to alter the level of nicotine accumulation. The effect of closer spacing would results in the lower nicotine concentration of tobacco leaves. The nitrogen content of the soil can be maintained by the introduction of nitrogenous fertilizer to the soil.

Health effect of cigarette smoke

A person's health risk is directly proportional to the length of time that a person continuous to smoke as well as the amount of smoke intake. the health risks of smoking are not uniform across all smokers; the risk varies according to the amount of tobacco smoked that is with those who smoke more at great risk. There is a wide variation in the nicotine content of tobacco in cigarettes, ranging from 7 mg/g to 23 mg/g in tobacco sampled from cigarettes from several countries.

The amount of nicotine absorbed from cigarettes depends on the intensity and volume of puffing, how much smoke is inheld and how many puffs are taken from the cigarette.

Typically, only between 0.5 mg to 3 mg of nicotine centers to the systematic circulation of smoker or pollute environment. This implies that cigarette smoke affects more passive smoker than active smokers. Studies on the chemical content of smoke released from burning tobacco indicates that cigarette smoke is composed of more than 4000 chemical compounds, approximately 500 of the compounds are found in vapor phase (including carbon monoxide, ammonia, nitrogen oxide, hydrogen cyanide and various hydrocarbons) and over 3500 are found in the particulate phase (including metals, tars, tobacco specific N-nitrosamines, poly nuclear aromatic hydrocarbons and tobacco non-specific N-nitrosamines) [2].

Generally, nicotine is a power full psychoactive agent that has a variety of central and peripheral nervous system effect, as well its effects go on the cardiovascular, endocrine, gastrointestinal and skeletal motor systems. The amount of nicotine in cigarettes are different in different cigarette brands. commonly the amount of nicotine in cigarettes are dependent on the amount of nicotine in the tobacco plant and the type of processing system during cigarette manufacturing.

MATERIALS AND METHODS

Apparatus and equipment

Double beam UV-Vis spectrometer with model (CE4400/UV-Vis) Perkin-Elmer spectrometer lambda19, with a wave length range 170-3200 nm, wave length accuracy 0.1 nm and slit width 1nm and 2 nm as required. Weighing balance (BP 210S, d=0.1mg, Max 210g made in Boulder Company), Quartez cuvette, volumetric flask, beaker, measuring cylinder, centrifuge model M212 Scott Behrens founder US company model. And the reaction temperature was attained using a water bath (GFL, D-30938 Bergwedel , Germany type).

Reagent and chemicals

Nicotine 98% (Riedel-de Haen AG Seelze-Hannover-62867, Germny), Potassium permanganate (pharmacos LTD, England), sodium hydroxide (Darmstadt, Germny), zinc acetate (Blulux laboratory reagent), potassium hexacyanoferrate (II), methanol was used as received. Distilled water was used throughout this study. Solution of nicotine (1000µg/ml), sodium hydroxide (10 M) potassium permanganate (0.125M), zinc acetate (1.22 M), and potassium hexacyanoferrate II (10.6% w/v) were prepared following reported procedures.

Samples

Nine cigarette brand samples, which are commonly sold in Ethiopia, were collected from the retailers' shops. Nyala, Nyala premium, delight and Gisella cigarette samples are all from Ethiopian products, Marlboro and Kent cigarette samples are both from USA products and Rothmans from UK then Royal from England products.

EXPERIMENTAL PART

Procedure for calibration graph of pure nicotine

In order to measure the concentration of nicotine present in nine different cigarette brands, it was necessary to construct calibration graph using pure nicotine. In doing so, measured pure nicotine was transferred in 100 ml volumetric flask and distilled water was added to the volumetric flask up to the mark followed by shaking by hand. A serious of solution was prepared using appropriate dilution.

To a 50 ml of serious of six volumetric flasks was added exactly 1 ml of 0.0125 M potassium permanganate followed by 2 ml of 6.25 M of sodium hydroxide solution. The flask was swirled gently to mix the contents. This was followed by addition of 25 ml pure nicotine of serious of solution to each flask and heat to 1000c for 8-minute cool to room temperature.

The concentration of nicotine in different cigarette sample was determined using UV-Vis spectrophotometer, since nicotine oxidized by potassium permanganate under basic condition producing a water-soluble green product (manganate ion) with a peak absorbance at 610 nm. The formation of green water-soluble product is due to the reduction of potassium permanganate (violate) to manganate which is green.

$$5C_{10}H_{14}N_2\,(Nicotine) + 4MnO_4 + 16H_2O \rightarrow 5C_6H_5NO_2 + 5C_4H_8O + 5NH_4^+ + 4MnO_4^{2-} + 17OH^-$$

Scheme, Oxidation of nicotine in alkaline media

The absorbance of green product (manganate ion) is directly proportional to the concentration of pure nicotine [7]. The peak absorbance of prepared nicotine standards was collected using UV-Vis spectrophotometer at 1 cm cell at a peak wave length of 610 nm against a reagent blank.

Procedure for nicotine extraction from cigarette samples

The extraction procedure used to determine the level of nicotine in nine different cigarette samples using double beam UV-Vis is spectrophotometer was taken from a literature procedure [8]. With a slight modification. To 50 ml beaker 10 ml of methanol was added to 50 mg of cigarette tobacco mixed well and stand for 30 min. 25 ml of distilled water and 1 ml of 2 M sodium hydroxide were added to a mixture with continues stirring. The mixture boiled for 1 minute using water bath and cool, then filtered using Whatman filter paper to 50 ml volumetric flask. The residue was washed two times using 10 ml distilled water and the filtrate combined. To a filtrate 1 ml of 1.22 M zinc acetate and 1 ml of 10.6% w/v potassium hexacyanoferrate II were added with continuous shaking and complete to the mark with distilled water. Centrifuge for 5 minute and transfer supernatant liquid to 50 ml beaker and 1 mg Fuller's earth was added then centrifuge. The residue was washed with 10 ml distilled water and centrifuge again. The residue with 5 ml of 0.01 M sodium hydroxide was heated for 2 minute and cool then filter with filter paper to a 50 ml volumetric flask. The residue was washed two times using 10 ml of distilled water and filtrate was combined then the volumetric flask completes to the mark with distilled water.

Procedure for nicotine determination in cigarette samples

To a 100 ml volumetric flask was added exactly 1 ml of 0.0125 M potassium permanganate solution. To the resulting mixture was added exactly 2 ml of 6.25 M sodium hydroxide solution. The flask was swirled gently to mix the contents. This was followed by addition of 50 ml nicotine solution prepared as per a literature procedure [8]. the volume of the solution was made up to about 80 ml by addition of distilled water. The resalting solution was heated in a water bath (100°C) for 8 min. the solution was then allowed to cool to room temperature. The volume was adjusted up to the mark (100 ml) using distilled water. The solution, well shaken with hand and the absorbance was collected using UV-Vis spectrophotometer at a 1 cm cell at 610 nm against a reagent blank.

RESULT AND DISCUSSION

Construction of calibration graph of pure nicotine solution

To determine the molar absorption coefficient (ε) of pure nicotine; solutions with different concentration were prepared and the absorbance of a pure nicotine for different concentrations were measured using UV-Vis spectrophotometer. The peak absorbance of each solution of pure nicotine in distilled water was collected at a maximum wave length of 610 nm. the graph of absorbance *versus* concentration of pure nicotine was constructed using SPSS software and concentration of pure nicotine, absorbance of pure nicotine and molar absorption coefficient (ε) were summarized in table 1.

Table 1

Data obtained for calibration curve for the determination of nicotine in cigarette

λ_{max}(nm)	Concentration of pure nicotine in µg/ml	Average absorbance of three trails	ε (m^2/µg)
610	1	0.036	3.6×10^{-6}
610	2	0.078	3.9×10^{-6}
610	4	0.146	3.65×10^{-6}
610	6	0.220	3.66×10^{-6}
610	8	0.302	3.77×10^{-6}
610	10	0.370	3.70×10^{-6}

From table 1. The absorbance verses concentration graph is a linear as shown below.

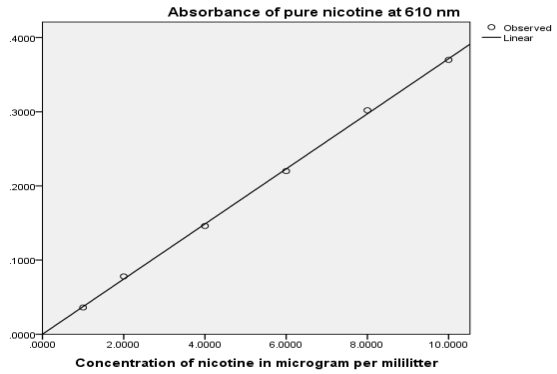

Figure 1) *Absorbance vs concentration graph for pure nicotine in distilled water*

The calibration curve of absorbance verses concentration graph is a linear as shown above. From the linear relationship between concentration and absorbance of pure nicotine, the molar absorption coefficients were easily determined by applying Beer-Lambert law.

$$A = \varepsilon Cl \qquad (1)$$

where

A is Absorbance

C is Concetration of pure nicotine

l is path length through the sample

ε is Molar absorptivity coefficient of pure nicotine

The equation representing the above linear relation between absorbance of pure nicotine with its concentration was derived using linear regression by the use of SPSS software version 23.

$$Y = 0.037X + 5.48 \times 10^{-5} \qquad (2)$$

Where Y is absorbance and X is concentration of pure nicotine.

Method of validation

The calibration graph of absorbance versus concentration of pure nicotine was constructed at the λ_{max} (610 nm) for a concentration rang of 1 µg/ml-10 µg/ml for pure nicotine in distilled water as shown figure 1. The standard deviation was found to be 0.0037 and the linear regression coefficient was 0.999. From figure 1, good linear relationships were observed for a wide concentration range. This indicated that absorbance is directly proportional to concentration as required by Beer-Lambert law.

Data interpretation

The weight of different sampled cigarettes tobacco varies depending on the length, moistures in cigarette and other factors. The average weight of sampled cigarettes without the filter and paper was (0.73 ± 0.09) g ranging from 0.65 ± 0.03 g (Royal) to 0.9 ± 0.06 g (Gissila). Concentration of nicotine in nine cigarettes samples were determined below using the standard calibration graph of pure nicotine solution. Concentration of nicotine in the sampled cigarettes were determined by determining the absorbance of extracted nicotine under oxidation of it with potassium permanganate in alkaline media using the UV-Vis spectrophotometer and by relating the absorbance of the solution with its concentration by applying the principle of Beer-Lambert law.

Equation 2 that used to determine the concentration of nicotine in the sampled cigarette was derived from the calibration graph of standard nicotine solution which was computed using SPSS software.

Determination of nicotine in cigarette brands

TABLE 2

Result of UV-Vis spectroscopic determination of concentration of nicotine in both Ethiopian and imported cigarette samples (mean ± SD) in mg/g, mg/ cigarette dry weight and percentage of nicotine by mass from the total tobacco weight. Mean value of triplicate analysis (n=3) is given

No.	Samples	Mass of tobacco in one cigarette (n=3)	Concentration of nicotine in mg of nicotine/gram of tobacco	Milligram of nicotine/cigarette	% of nicotine by dry weight of tobacco
1	Nyalla	0.73 ± 0.03	8.93 ± 0.35	12.23	0.89 ± 0.035
2	Nyalla premium	0.72 ± 0.10	12.27 ± 1.46	17.04	1.227 ± 0.146
3	Delight	0.75± 0.06	8.47 ± 0.45	11.29	0.847 ± 0.045
4	Rothmans	0.73 ± 0.04	11.27 ± 1.35	15.44	1.127 ± 0.135
5	Marlboro	0.66 ± 0.04	16.4 ± 1.25	24.85	1.640 ± 0.125
6	Ghamdane	0.72 ± 0.08	9.67 ± 1.30	13.43	0.967 ± 0.130
7	Royal	0.65 ± 0.03	8.89 ± 0.25	13.68	0.889 ± 0.025
8	Gisela	0.90 ± 0.06	11.0 ± 1.56	12.22	1.100 ± 0.156
9	Kent	0.70 ± 0.05	6.73 ± 0.87	9.61	0.673 ± 0.087
	Mean	0.73 ± 0.09	10.51 ± 2.92	14.4	1.051 ± 0.292

Table 3

concentration of nicotine (mean ± SD) in mg/gram, mg/cigarette and in percent of nicotine per dry tobacco in Ethiopia cigarette brands (n=3)

No	Samples	Mass of tobacco in one cigarette (n=3)	Concentration of nicotine in mg of nicotine/gram of tobacco	Milligram of nicotine/cigarette	% Of nicotine by dry weight of tobacco
1	Nyalla	0.73 ± 0.03	8.93 ± 0.35	12.23	0.89 ± 0.035
2	Nyalla premium	0.72 ± 0.10	12.27 ± 1.46	17.04	1.227 ± 0.146
3	Delight	0.75± 0.06	8.47 ± 0.45	11.29	0.847 ± 0.045
4	Gisela	0.90 ± 0.06	11.0 ± 1.56	12.22	1.100 ± 0.156
	Mean	0.77 0.099	10.17 ± 2.16	13.19	1.017 ± 0.216

Table 4

concentration of nicotine (mean ± SD) in mg/gram, mg/cigarette and in percent of nicotine per dry weight of tobacco of some imported cigarette brands (n=3)

No.	Samples	Mass of tobacco in one cigarette (n=3)	Concentration of nicotine in mg of nicotine/gram of tobacco	Milligram of nicotine/cigarette	% Of nicotine by dry weight of tobacco

1	Rothmans	0.73 ± 0.04	11.27 ± 1.35	15.44	1.127 ± 0.135	
2	Marlboro	0.66 ± 0.04	16.4 ± 1.25	24.85	1.640 ± 0.125	
3	Ghamdane	0.72 ± 0.08	9.67 ± 1.30	13.43	0.967 ± 0.130	
4	Royal	0.65 ± 0.03	8.89 ± 0.25	13.68	0.889 ± 0.025	
5	Kent	0.70 ± 0.05	6.73 ± 0.87	9.61	0.673 ± 0.087	
	Mean	0.692 ± 0.036	10.59 ± 3.49	15.3	1.059 ± 0.349	

The findings indicated that the core component of cardiac rehabilitation after heart valve surgery includes patient assessment, modification or adjustment of physical activity counselling, exercise training, diet/nutritional counselling, smoking cessation and psychological management [4,8]. As can be seen from the above tables concentration of nicotine varies strongly through different cigarette brands. Average concentration of nicotine in different cigarette brands in three trials Nyalla (8.93 ± 0.35 mg/g), Nyalla premium (12.27 ± 1.46 mg/g), Delight (8.47 ± 0.45 mg/g), Rothman (11.27 ± 1.35 mg/g), Marlboro (16.4 ± 1.25 mg/g), Ghamdane (9.67 ± 1.30 mg/g), Royal (8.89 ± 0.25 mg/g), Gisilla (11.0 ± 1.56 mg/g), Kent (6.73 ± 0.87 mg/g) and the whole total mean of nicotine in nine cigarette brand was found to be 10.51 ± 2.92 mg/g (Tables 2-4).

Concentration of nicotine was found to be 16.4 ± 1.25 mg/gram (highest) in Marlboro and 6.73 ± 0.87 mg/gram (lowest) in Kent that is the concentration of nicotine in Marlboro is more than two times the concentration of nicotine in Kent cigarette brands.

Independent t-test was applied to check whether there is a statistically significant difference in nicotine content in mg/g between Ethiopian and imported cigarette brands. Ethiopian cigarette brand (mean=10.17, SD=2.16), imported cigarette brands (mean=10.59, SD=3.49), this confirmed that there is no statistically significant difference in nicotine content between Ethiopian and imported cigarette brands ($P>0.05$). But nicotine content has statistically significant difference between Ethiopian cigarette (mean=13.19), and imported cigarette brand (mean=15.30) $p<0.05$, when expressed in mg/cigarette. This difference is due to the difference in mass of tobacco blend that packed in a cigarette (length of cigarette, packing intensity, moisture content of tobacco blend etc). The difference in nicotine concentration of different cigarette brands may be due to the following factors. The type of tobacco used, the position of tobacco leaves used, topography of tobacco that grow, the type of curing or drying process used, the amount and type of additives used during processing of cigarettes made to have different concentration of nicotine in different cigarette brand [9].

Level of nicotine in the same tobacco plants also varies due to position of the leaf. The level of nicotine in Virginia and other types of tobacco leaves increase upwards, that is the level of nicotine in tobacco leaves was found to have, 1.07 ± 0.04% (lung), 1.22±0.06% (cutter), 2.87 ± 0.10% (leaf) and 3.60 ± 0.08% (tip). Tobacco blends used to manufacture a particular cigarette brand has its own proportionalities of tobacco leaves by position; this made to have different amount of nicotine in different cigarette brands.

CONCLUSION

The level of nicotine in nine commercially available cigarette brands were determined by UV-Vis spectroscopic photometer. The analysis of level of nicotine in Ethiopian cigarette brand and imported cigarette brand demonstrated that highest level of nicotine was found in imported cigarette brand (Marlboro=16.4 ± 1.25 mg/g), the lowest level of nicotine also found in imported cigarette brand (Kent=6.73 ± 0.87 mg/g). This also confirmed that Ethiopian cigarette brand contain a nicotine content in between the highest and lowest nicotine content of imported cigarette brands. Finally, even if smoking is not recommended, smokers are better to smoke Kent and delight cigarette which contain small nicotine content that Marlboro and Rothman cigarette brand which have great nicotine content.

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