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Extraction and Estimation of Nicotine Present in Different Tobacco products

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Abstract

Nicotine or 3-(1-methyl-1-2-pyrrolidinyl) pyridine is an alkaloid found in the nightshade family of plants (Solanaceae), predominantly in tobacco, and in lower quantities in tomato, eggplant and in green pepper. Nicotine is a hygroscopic, colourless to yellow-brown, oily liquid that is readily soluble in alcohol, ether or light petroleum. It is miscible with water in its base form between 60 ℃ and 210℃. As a nitrogenous base, nicotine forms salts with acids that are usually solid and water-soluble. Its flash point is 95 ℃ and its auto-ignition temperature is 244℃. The objective of this experiment was to extract nicotine present in different tobacco products and to do a comparative study. Nicotine was extracted using liquid - liquid extraction method and the organic solvent used in the extraction process was diethyl ether. Different tobacco products like non-filtered tobacco (beedi/bidi), cigarettes, and chewing tobacco were analyzed. The study concludes that the nicotine content in tobacco contained in country cigarettes (beedi/bidi) is higher compared to the content in company manufactured branded cigarettes. The nicotine content in commercially available chewing tobacco products was found to be lower than in the smoking form of tobacco, but the average daily consumption made it comparable to the smoking form.

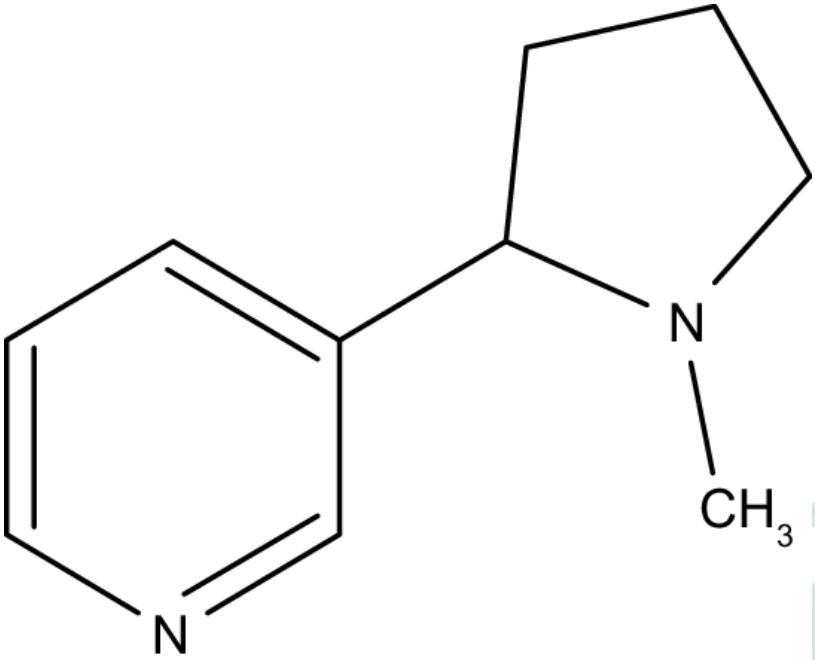
***Key words*: Nicotine, Solanaceae, Liquid-liquid extraction, Tobacco, Cigarettes.**

INTRODUCTION

Nicotine or 3-(1-methyl-1-2-pyrrolidinyl) pyridine is an alkaloid found in the nightshade family of plants (Solanaceae), predominantly in tobacco, and in lower quantities in tomato, eggplant, and in green pepper. Nicotine is a hygroscopic, colourless to yellow-brown, oily liquid that is readily soluble in alcohol, ether or light petroleum. It is miscible with water in its base form between 60 ℃ and 210℃. As a nitrogenous base, nicotine forms salts with acids that are usually solid and water-soluble. Its flash point is 95 ℃ and its auto-ignition temperature is 244℃. Nicotine is readily volatile (vapour 5.5 Pa at 25℃) and dibasic ($K_{b1} = 1 \times 10^{-6}$ $K_{b2} = 1 \times 10^{-11}$) (Robert L. Metcalf, 2007)[39].

Nicotine is optically active, having two enantiomeric forms. The naturally occurring form of nicotine is levorotatory with a specific rotation of $[\alpha]_D = -166.4°$ ((−) -nicotine). The dextrorotatory form, (+)-nicotine is

physiologically less active than (−)-nicotine. (−) -nicotine is more toxic than (+)-nicotine Gause, G. F. (1941). The salts of (+)-nicotine are usually dextrorotatory[13].



CHEMICAL STRUCTURE OF NICOTINE

Nicotine is named after the tobacco plant ***Nicotiana tabacum,*** which in turn is named after the French ambassador in Portugal, Jean Nicot de Villemain, who sent tobacco and seeds to Paris in 1560, presented to the French King, (Rang H. P. *et. al.*;2007)[38] who promoted their medicinal use. Nicotine was first isolated from the tobacco plant in 1828 by physician Wilhelm Heinrich Posselt and chemist Karl Ludwig Reimann of Germany, who considered it a poison[16]. Its chemical empirical formula was described by Melsens in 1843,[27] its structure was discovered by Adolf Pinner and Richard Wolffenstein in 1893,[34] and it was first synthesized by Amé Pictet and A. Rotschy in 1904[32].

Nicotine constitutes approximately 0.6 -3.0% of the dry weight of tobacco.[44] Less than one millionth of that concentration (2−7 µg/kg) is found in edible Solanaceae such as eggplants and tomatoes.[5] It functions as an anti-herbivore chemical; consequently, nicotine was widely used as an insecticide in the past.[47] Nicotine is highly addictive.[18] An average cigarette yields about 2 mg of absorbed nicotine, and in lesser doses of that order, the substance acts as a stimulant in mammals, while high amounts (50−100 mg) can be harmful.[26] This stimulant effect is a contributing factor to the addictive properties of tobacco smoking. Nicotine's addictive nature includes psychoactive effects, drug-reinforced behaviour, compulsive use, and relapse after abstinence, physical dependence and tolerance.[4]

Nicotine is both a stimulant a relaxant.[8] First causing a release of glucose from the liver and epinephrine (adrenaline) from the adrenal medulla, it causes stimulation. When a cigarette is smoked, nicotine-rich blood passes from the lungs to the brain within seven seconds and immediately stimulates nicotinic acetylcholine receptors; this indirectly promotes the release of many chemical messengers such as acetylcholine, norepinephrine, epinephrine, arginine vasopressin, serotonin, dopamine, and beta-endorphin in parts of the brain.[35][36] The release of epinephrine (adrenaline) causes an increase in heart rate, blood pressure and respiration, as well as higher blood glucose levels.[9] Nicotine also extends the duration of positive effects of

dopamine and increases the sensitivity of the brain's reward system to rewarding stimuli.[7][21] Most cigarettes contain 1−3 milligrams of inhalable nicotine.[10]

Nicotine also has an affinity for melanin-containing tissues due to its precursor function in melanin synthesis or due to the irreversible binding of melanin and nicotine. This has been suggested to underlie the increased nicotine dependence and lower smoking cessation rates in darker pigmented individuals. As nicotine enters the body, it is distributed quickly through the bloodstream and crosses the blood−brain barrier reaching the brain within 10−20 seconds after inhalation.[23] The elimination half-life of nicotine in the body is around two hours.[2] Nicotine is metabolized in the liver by cytochrome p450 enzymes which selectively metabolizes (S)-nicotine. A major metabolite is cotinine which is an active metabolite of nicotine that remains in the blood for 18−20 hours, making it easier to analyse due to its longer half-life.[3]
The amount of nicotine absorbed by the body from smoking can depend on many factors, including the types of tobacco, whether the smoke is inhaled, and whether a filter is used. However, it has been found that the nicotine yield of individual products has only a small effect (4.4%) on the blood concentration of nicotine[40] suggesting "the assumed health advantage of switching to lower-tar and lower-nicotine cigarettes may be largely offset by the tendency of smokers to compensate by increasing inhalation".

Extraction method:

The extraction was done based on liquid- liquid extraction technique. Liquid-liquid extraction also known as solvent extraction and partitioning is a method to separate compounds based on their relative solubilities in two different immiscible liquids, usually water and an organic solvent. Nicotine is hygroscopic, oily liquid that is miscible with water in its base form. As a nitrogenous base, nicotine forms salts with acids that are usually solid and water soluble. The extraction depends on isolation of base by dissolving the cigarettes in NaOH. Nicotine is then extracted from the filtrate by ether. After evaporation of ether nicotine oil is obtained. To obtain nicotine crystals, saturated solution of picric acid was added to form nicotine di picrate yellow crystals.[31]

Lassaigne's test is the most reliable test for the detection of nitrogen. In order to check for the presence of a pyridine and pyrrolidine ring with nitrogen atom attached to it the sample extract was subjected to Lassaigne's test. The carbon and nitrogen present in the organic compound on fusion with sodium metal gives Sodium Cyanide (NaCN) soluble in water. This is converted into sodium ferrocyanide by the addition of sufficient amount of Ferrous Sulphate. Ferric ions generated during the process reach with ferrocyanide to form Prussian blue precipitate of ferric ferrocyanide.

Iodoplatinate test is a general test for alkaloids. It involves dissolving the sample in two drops of 2 M hydrochloric acid and 2-3 mL of the Iodoplatinate reagent (solution of platinic chloride and potassium iodide) which gives a violet precipitate suggesting the presence of an alkaloidal base precipitated as the alkaloid−iodoplatinate complex.

Dragendorff's reagent is a color reagent test used for the analysis alkaloid present in the sample. Alkaloids, if present in the solution of sample, will react with Dragendorff's reagent and produce an orange, red−orange or brown−orange precipitate. The orange precipitate suggests the presence of a pyrollidine alkaloid, precipitated as the alkaloidal bismuth iodide.

Sanchez (1922) described a colour test for nicotine in which a rose red colour is produced when nicotine is added to a solution of vanillin in concentrated HCI.[29]

Uses of nicotine

Medical

The primary therapeutic use of nicotine is in treating nicotine dependence in order to eliminate smoking with the damage it does to health. Controlled levels of nicotine are given to patients through gums, dermal patches, lozenges, electronic/substitute cigarettes or nasal sprays in an effort to wean them off their dependence. Studies have found that these therapies increase the chance of success of quitting by 50 to 70%[45] though reductions in the population as a whole have not been demonstrated.[33]

Enhancing performance

Nicotine is frequently used for its performance-enhancing effects on cognition, alertness, and focus.[20] A meta-analysis of 41 double-blind, placebo-controlled studies concluded that nicotine or smoking had significant positive effects on aspects of fine motor abilities, alerting and orienting attention, and episodic and working memory.[15] A 2015 review noted that stimulation of the α4β2 nicotinic receptor is responsible for certain improvements in attentional performance;[43] among the nicotinic receptor subtypes, nicotine has the highest binding affinity at the α4β2 receptor (k_i=1 nM), which is also the biological target that mediates nicotine's addictive properties.[28]

Recreational drug

Nicotine is commonly consumed as a recreational drug for its stimulant effects.[6] Recreational nicotine products include chewing tobacco, cigars, cigarettes, e-cigarettes, snuff and pipe tobacco.

REVIEW OF LITERATURE

Mabroukah Al-Darmoon et.al in 2015 extracted nicotine from three local produced tobacco and ten international brands of locally sold tobacco. The concentrations of nicotine (mg nicotine/g cigarette) in all tobacco brands studied were measured using ultraviolet-visible (UV-Vis) spectrophotometer at wavelength of λ=602nm. The pattern of nicotine concentrations in Libyan chews were followed green>dry>Ziliten, while were varied in the international tobacco brands. The nicotine content in Libyan tobacco cigarette brand (Al-Riadei) was found to contain on average 4.20 mg nicotine/g cigarette, while the international tobacco brands were found to contain from 3.85E-02 to 1.40E0l mg nicotine/g cigarette. The highest nicotine concentration was found in the international brand named Capital, while the lowest was found in the international brand named Business. The findings in this work showed that the nicotine contents in all brands studied were under a lethal dosage for adult-human, but higher than the significant impact on nicotine intake.[24]

Arie Febrianto Mulyadi et.al in the year 2013 found that the properties of nicotine is soluble to some types of solvents. This is the reason for the extraction of nicotine by using a solvent extraction method. In the extraction of nicotine as an alkaloid in tobacco, ether and petroleum ether solvent is advantageous because it is selective in dissolving the alkaloid substances. Using the right combination of ether and petroleum ether optimizes the time of extraction and the yield of nicotine on nicotine extraction process. The result of the research revealed that the addition of ether and petroleum ether solvent significantly affect the response time of extraction and yield. Predicted results obtained optimal solution was the addition of 59.46 ml of ether and 30.12 ml of petroleum ether. The lowest possible values for the extraction time was 477.343 seconds and the highest is 887.623 seconds.[1]

Sahar Taghavi et.al in the year 2012 used fourteen popular imported brands and nine popular domestic brands of cigarettes and three available brands of tobaccos for investigation of the amounts of nicotine content. Nicotine was extracted from each cigarette and tobacco samples and was analyzed by high performance liquid chromatography (HPLC) method. The amount of nicotine in each cigarette was from 6.17 to 12.65 mg (1.23 ±

0.15 percent of tobacco weight in each cigarette) in domestic cigarettes. It was between 7.17-28.86 mg (1.80 ± 0.25 percent of tobacco weight in each cigarette) for imported cigarette, and between 30.08- 50.89 mg (3.82 ± 1.11 percent) for the pipe nicotine. There was significant difference in nicotine amount between imported and domestic brands of cigarettes. There was also no significant difference in nicotine content between light and normal cigarettes in imported brands.[42]

Sujatha S Reddy and KH Shaik Hyder Ali in year 2008 used commercially available cigarettes, bidis, and pan masalas (chewable tobacco) for the study. Nicotine was estimated using gas-liquid chromatography. The analyses showed relatively higher levels of nicotine in tobacco from bidis (26.9 mg gm) as compared to cigarettes (15 mg/gm); the difference is statically significant (P <0.001). The nicotine concentration of tobacco from filtered cigarettes averaged 14.5 mg/gm whereas unfiltered cigarettes averaged 15.6 mg/gm; the difference was not statically significant (P >0.01). Nicotine concentration in chewing tobacco was 3.4 mg/gm. The study concludes that the nicotine content of Indian brands of smoking tobacco was slightly high compared to other international brands. Higher concentration of nicotine was found in bidis compared to cigarettes. The nicotine content in commercially available chewing tobacco products was found to be much lower than in the smoking form of tobacco, but the average daily consumption made it comparable to the smoking form.

MATERIALS AND METHODS

MATERIALS

Tobacco samples, Beakers, Buchner funnel, Filter paper, Conical flask, 250ml Separating funnel, Stand, Water bath, Measuring cylinder, Weighing balance, Tongs, Bunsen burner, Test tubes, Test tube stand, mortar and pestle, Sodium fusion tubes.

CHEMICALS REQUIRED

Sodium hydroxide, Diethyl ether, Anhydrous potassium carbonate, Picric acid, Methanol, Sodium metal, Ferrous sulphate, Ferric chloride, Sulphuric acid, Platinic chloride, Potassium iodide, Hydrochloric acid. Vanillin, Bismuth subnitrate, Bismuth potassium iodide.

METHODOLOGY

EXTRACTION OF NICOTINE

STEPS INVOLVED

STEP 1: Five different tobacco samples were taken and one gram of each sample was weighed using a weighing balance and then transferred into a beaker. [Fig-1]

STEP 2: 100ml of 5% NaOH solution was prepared by dissolving 5 gram of NaOH in 100ml of water. The above solution was mixed with the sample in the beaker and stirred well for 15 min. The solution was then filtered using Buchner funnel. The cigarettes were again transferred to a beaker and 30ml of distilled water was added, stirred and filtered again. [Fig-2]

STEP 3: The filtrates were collected together (re-filtered if there was any impurity). The filtrates were transferred into a separating funnel and extracted by 25ml of Diethyl ether. The extraction was repeated 3 times and the 4 filtrates were collected in a beaker. [Fig-3]

STEP 4: The extract was dried by using 2 grams of anhydrous potassium carbonate, filtered and allowed the ether to be evaporated on water bath. (Extra heat was avoided because nicotine is hydrolysed by extreme heating). [Fig-4]

STEP 5: After evaporation of ether 4ml of methanol was added to dissolve the resulted oil. [Fig-5]

STEP 6: 10ml of saturated picric acid solution was added to the beaker. Which was then cooled in an ice bath to precipitate the nicotine di picrate crystals. [Fig-6]

STEP 7: The crystals were allowed to dry and then weighed. [Fig-7]

QUALITATIVE ANALYSIS OF NICOTINE

LASSAIGNE'S TEST (TEST FOR NITROGEN)

PREPRATION OF SODIUM FUSION EXTRACT

A small piece of Sodium metal (about a quarter size of a pea) was taken in a sodium fusion tube and warmed gently until it melted and formed a shining globule. A small amount of the sample was added to the fusion tube and heated gently till the reaction with sodium metal ceases and then strongly till the fusion tube became red hot. The red hot tube was plunged in a mortar containing 10ml of distil water. The solution was filtered and boiled gently for five minutes. The filtrate was collected and cooled and used as the stock solution or fusion extract.

EXPERIMENT: To a portion of the filtrate about $^1/_2$ml of freshly prepared solution of ferrous sulfate was added along with one drop of ferric chloride The solution was heated for short time, cooled and a few drops of sulfuric acid was added.

$Na + C + N \rightarrow NaCN$

$6NaCN + FeSO_4 \rightarrow Na_4[Fe(CN)_6] + Na2SO_4$
Sodium Ferrocyanide

$Na_4[Fe(CN)_6] + Fe^{3+} \rightarrow Fe_4[Fe(CN)_6]_3$
Ferric Ferricyanide

IODOPLATINATE TEST (general test for alkaloids)

Reagent: 2 mL of a 5% (w/v) solution of platinic chloride and 5 g of potassium iodide was added to 98 mL of water and dissolved well.

Experiment: The sample was dissolved in two drops of 2 M hydrochloric acid and 2 to 3 mL of the above mentioned reagent and dilute to 10 mL with water.

DRAGENDORFF'S TEST (a general reagent for nitrogenous bases)

Reagent: 1 g of bismuth subnitrate was dissolved in 3 mL of 10 M hydrochloric acid with the aid of heat and diluted to 20 mL with water. 1 g of potassium iodide was added to the mixture.

Experiment: The sample was dissolved in three drops of 2 M hydrochloric acid and 2 to 3 mL of the reagent and dilute to 10 mL with water.

COLOUR TEST FOR NICOTINE

The sample was added to a solution of vanillin in concentrated HCl.

OBSERVATION

EXTRACTION OF NICOTINE

SAMPLE 1:

Weight of empty beaker (w_1 g) = 55.523g

Weight of beaker + sample (w_2 g) = 55.546g

Mass of the sample (w_2 - w_1) = 55.546 — 55.523 = 0.023g

The amount of nicotine extracted from one gram of tobacco sample was found to be 23 mg

SAMPLE 2:

Weight of empty beaker (w_1 g) = 51.827g

Weight of beaker + sample (w_2 g) = 51.842g

Mass of the sample (w_2 - w_1) = 51.842 – 51.827 = 0.015g

The amount of nicotine extracted from one gram of tobacco sample was found to be 15 mg

SAMPLE 3:

Weight of empty beaker (w_1 g) = 49.667g

Weight of beaker + sample (w_2 g) = 49.675g

Mass of the sample (w_2 – w_1) = 49.675 – 49.667 = 0.008g

The amount of nicotine extracted from one gram of tobacco sample was found to be 8 mg

SAMPLE 4:

Weight of empty beaker (w_1 g) = 49.88g

Weight of beaker + sample (w_2 g) =49.893g

Mass of the sample (w_2-w_1) = 49.893 – 49.88 = 0.013g

The amount of nicotine extracted from one gram of tobacco sample was found to be 13 mg.

SAMPLE 5:

Weight of empty beaker (w_1 g) = 48.781 g

Weight of beaker + sample (w_2 g) = 48.799g

Mass of the sample (w_2 - w_1) = 48.799 – 48.781 = 0.018g

The amount of nicotine extracted from one gram of tobacco sample was found to be 18 mg.

Lassaigne's Test

A Prussian blue colour is formed [Fig-8]

Iodoplatinate Test

A violet coloured precipitate was formed [Fig-9]

Dragendorff Test

An orange precipitate was formed [Fig-10]

Colour test for Nicotine

A rose red colour was formed [Fig-11]

PLATE- 1

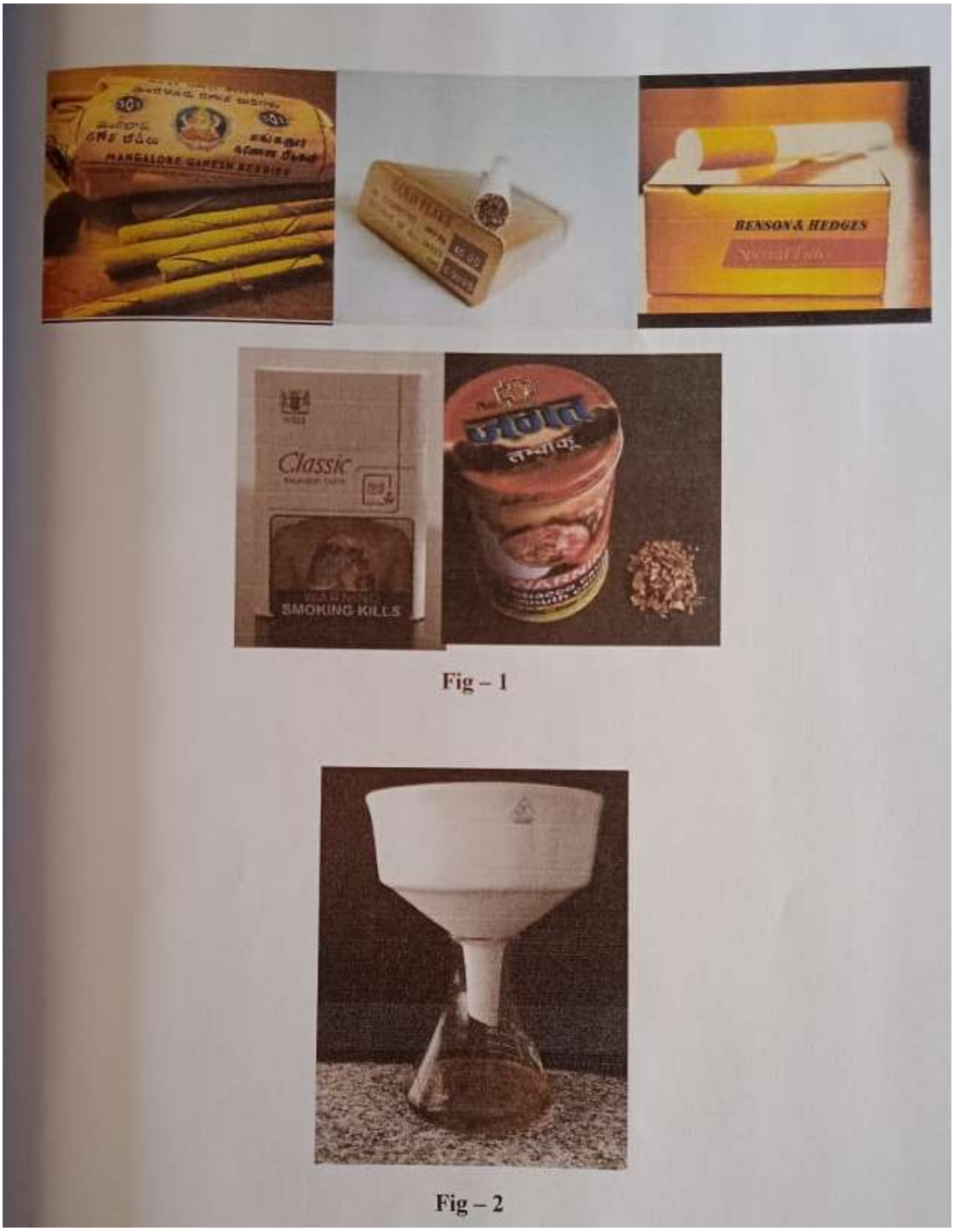


Fig – 1

Fig – 2

Fig- 1: Tobacco samples (A, B, C, D, and E)

Fig-2: Extraction using NaOH

PLATE – 2

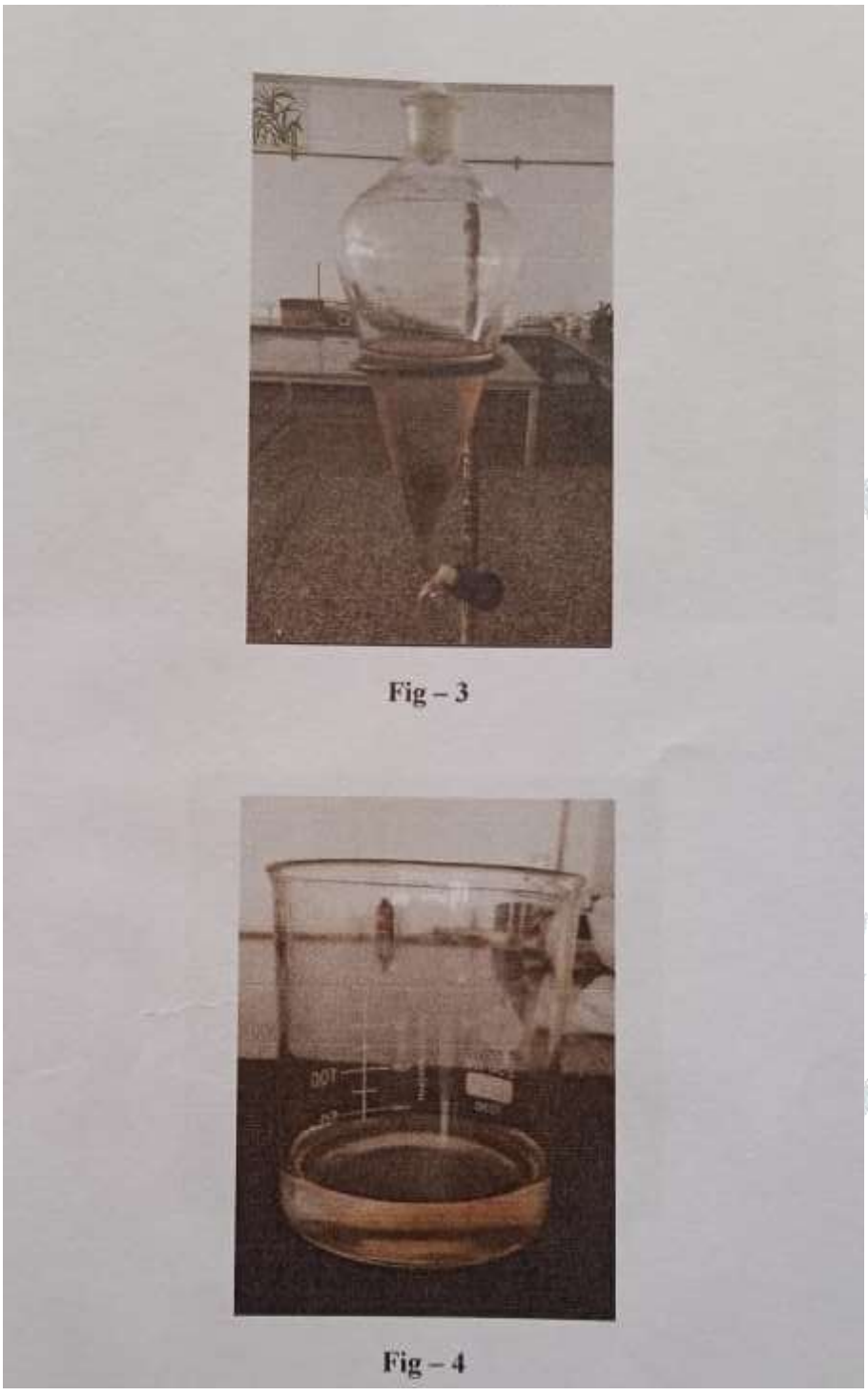


Fig – 3

Fig – 4

Fig – 3: Ether extraction

Fig – 4: Evaporation of Ether

PLATE – 3

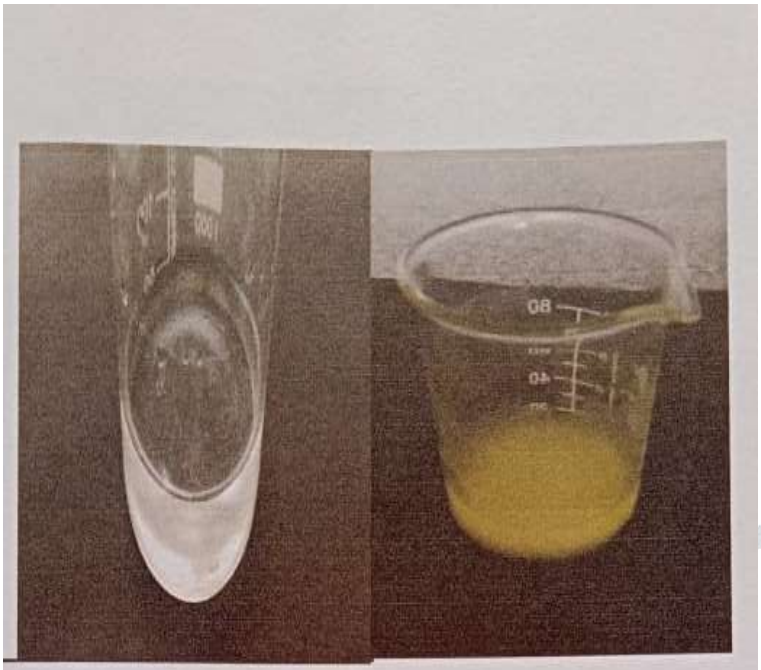


Fig – 5

Fig – 6

Fig – 7

Fig – 5: Addition of methanol

Fig – 6: Precipitation of Nicotine dipicrate

Fig – 7: Crystallization

PLATE – 4

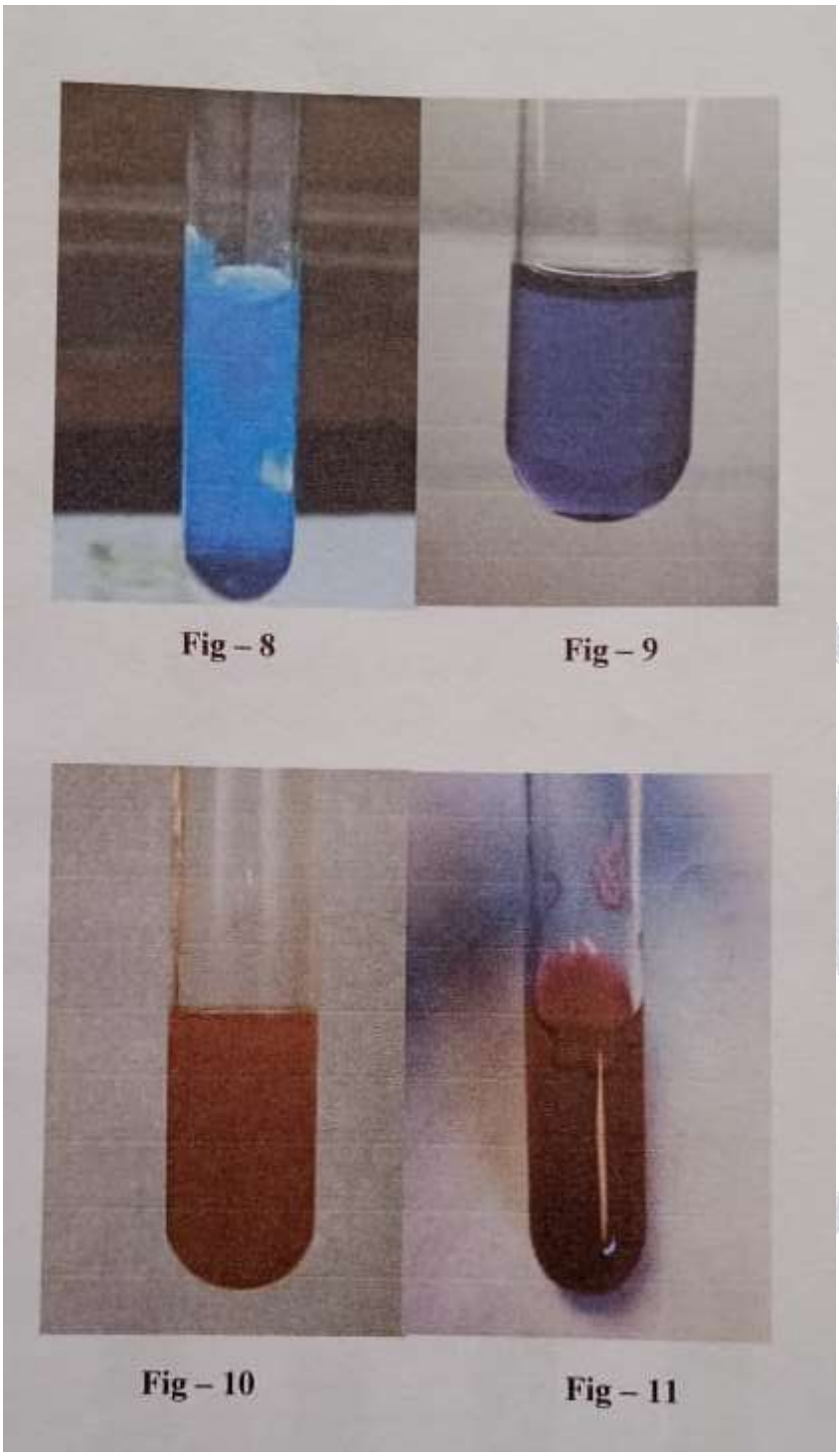


Fig – 8: Lassaigne's Test

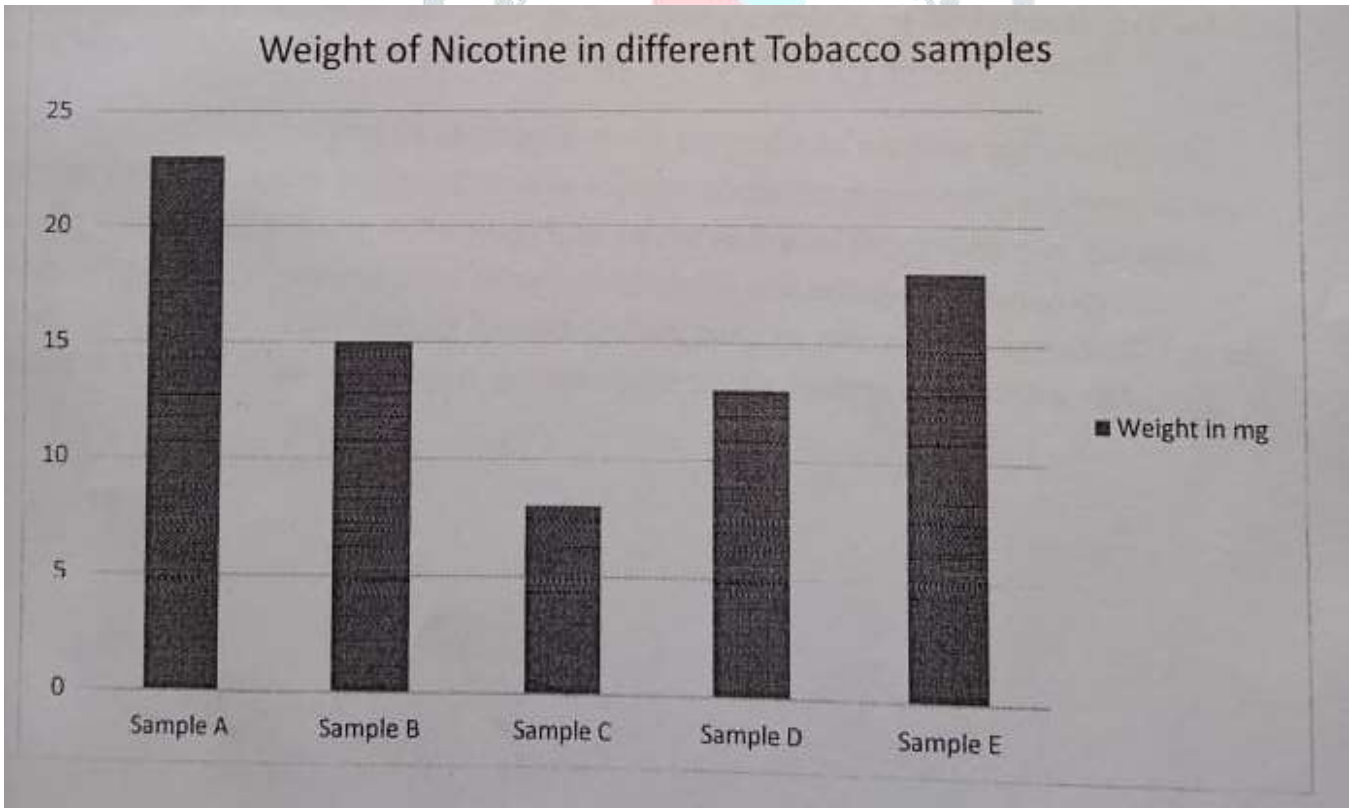
Fig – 9: Iodoplatinate Test

Fig – 10: Dragendorff Test

Fig – 11: Colour test for Nicotin

RESULT

Sl.No.	Tobacco samples	Weight
1	A (beedi/bidi)	23 mg/g
2	B (Gold Flake)	15 mg/g
3	C (Benson and Hedges)	8 mg/g
4	D (Classic)	13 mg/g
5	E (Jagat chewing tobacco)	18 mg/g



DISCUSSION

The objective of this experiment was to extract nicotine present in different tobacco products and to do a comparative study. Nicotine was extracted
using liquid - liquid extraction method and the organic solvent used in the extraction process was Diethyl ether. The extraction procedure was repeated until a concordant value

of the weight was obtained. The color of the sample extracted was yellow which was due to the addition of picric acid. Different tobacco products like non – filtered tobacco (beedi/bidi), cigarettes, and chewing tobacco were analyzed.

The beedis/bidis tested contained less tobacco than other cigarettes. However, their tobacco contained significantly higher concentrations of nicotine than the tobacco of commercial mg/g, whereas it averaged 23 mg/g in smoking tobacco (beedi/bidi). That indicates that the nicotine concentrations in the smoking form of tobacco was found to be relatively higher compared to that in chewing tobacco; however, this is largely compensated for by the greater intake of chewing tobacco. According to Centre for Disease Control (CDC), chewing tobacco used 7-8 times a day may be equivalent to smoking 30-40 cigarettes per day.

The nicotine concentration of the other cigarette samples averaged between 8 mg/g to 15 mg/g. Some brands had a higher nicotine content than others, indicating that tobacco types or blends and tobacco casings can be used to manipulate the nicotine content and the nicotine delivery of cigarettes. The difference in the nicotine concentration between these samples, can perhaps, be attributed to difference in the technique employed for estimation of nicotine, plant variety, cultivation, curing methods, and the design of the tobacco product.

A series of test were performed in order to check the purity of nicotine in the extracted sample. Lassaigne's test was performed in order to check the presence of nitrogen in the sample where the formation of Prussian blue colour indicated the presence of nitrogen. Iodoplatinate Test which is a general test for alkaloids and nitrogenous heterocyclic compounds gave a violet coloured precipitate indicating its presence. Dragendorff Test and Colour test for Nicotine also gave a positive result for the presence of nicotine alkaloid.

CONCLUSION

The study concludes that the nicotine content in tobacco contained in country cigarettes (beedi/bidi) is higher compared to the content in company manufactured branded cigarettes.

The nicotine content in commercially available chewing tobacco products was found to be lower than in the smoking form of tobacco, but the average daily consumption made it comparable to the smoking form.

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