Project report

on

Analysis of Nicotine Content in Different Brands of Smoking and Chewing Tobacco Products

Submitted in Partial Fulfilment of the Requirement for the Degree of M.Sc. Forensic Science

Submitted by

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M.Sc. Forensic Science (IVth Semester)

Under the Supervision of

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May 2020

GALGOTIAS UNIVERSITY
(Established under Galgotias University Uttar Pradesh Act No. 14 of 2011)

School of Basic and Applied Science

CERTIFICATE

This is to Certify that Mr./Ms. **Kavita Kumari** has carried out his/her project work entitled "**analysis of nicotine content in different brands of smoking and chewing tobacco products**" under my supervision. This work is fit for submission for the award of Master Degree in Forensic Science.

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विश्वविद्यालय
(CREATING KNOWLEDGE)

GALGOTIAS
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No. 12 of 2018)

Dr. L. P. Chandravanshi
(Ph.D.)
Assistant Professor & Head
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NO. DIVFSC/MSC/DEC/11

To
DIRECTOR CODON BIOTECH Pvt. Ltd
Noida

Date: 06/01/2020

Subject: Training for 4 months in Analytical Chemistry Division.

Dear Sir,

With regards to the above-mentioned subject, I am writing in seeking Training for a period of 4 months for Ms. Kavita. She is the final year student of M.Sc. Forensic Science at Division of Forensic Science, School of Basic and Applied Sciences, Galgotias University, Greater Noida, U.P.

She is very keen to undergo Training for a period of 4 month in the CODON BIOTECH Pvt. Ltd. I request you to kindly allow her to work for a period of 4 months.

Your timely guidance and support will help the students in enriching the knowledge in Forensic Science.

Thanks

Lalit P Chandravanshi Ph. D
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CERTIFICATE

This is to certify that the Project entitled "**ANALYSIS OF NICOTINE CONTENT IN DIFFERENT BRANDS OF SMOKING AND CHEWING TOBACCO PRODUCTS**" submitted in partial fulfillment for the award of the Degree of **M.Sc. (FORENSIC SCIENCES)** from Galgotias University, Greater Noida, is a record of research work carried out by **MS. KAVITA KUMAR** from 13th January 2020 to 16th March 2020 under my guidance and supervision.

All the help and assistance received during the course of this investigation have been duly acknowledged.

Tripti Bhatnagar

Dated : 07/05/2020
Place : Noida
No : CBPL2020/092

(Dr. Tripti Bhatnagar)
Managing Director
Codon Biotech Pvt. Ltd.
Noida

CANDIDATE DECLARATION

I hereby declare that the dissertation entitled "**analysis of nicotine content in different brands of smoking and chewing tobacco products**" submitted by me in partial fulfillment for the degree ofM.Sc. in Forensic Science to the Division of Forensic Science, School of Basic and Applied Science, Galgotias University, Greater Noida, Uttar Pradesh, India is my original work. It has not been submitted in part or full to this University of any other Universities for the award of diploma or degree.

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I am highly indebted to Dr. Mamta (Guide) for their guidance and constant supervision as well as for providing necessary information regarding the project & also for their support in completing the project.

I would like to express my gratitude towards my co-supervisor Dr. Tripti Bhatnagar (Managing Director, CODON Biotech Laboratory) for their kind co-operation and encouragement which help me in completion of this project.

I would like to express my special gratitude and thanks to our H.O.D(Dr. Lalit Chandravanshi) for giving me such opportunity to do project work.

My thanks and appreciations also go to my friends in developing the project and people who have willingly helped me out with their abilities.

(Signature)

Kavita Kumari

TABLE OF CONTENTS

List of Images

List of Abbreviations

1. Thin Layer Chromatography- TLC
2. Retention Factor- Rf
3. Methyl Jasmonate- MeJA
4. Jasmonate Inducible Alkaloid Transporter- JAT2
5. Nicotine Uptake Permease- NUP1
6. Milligram- mg
7. Hours- hrs.
8. Milligram of something per kilogram- mg/kg^{-1}
9. Nanogram of something per gram- ng/g
10. Hubble-Bubble- HB
11. Electronic Cigarettes- EC
12. Smoking Tobacco- ST
13. Potassium Hydroxide- KOH
14. Nanogram- ng
15. Rotation per minute- rpm
16. Minutes- min

PROJECT SUMMARY

The purpose of this study is to estimate the nicotine content in different brands of smoking and chewing tobacco products. Nicotine is a highly soluble natural alkaloid obtained from tobacco plants (*Nicotiana*). *Nicotiana rustica* and *Nicotiana tabacum* are used to manufacture various smoking, chewing, and sniffing tobacco products. Different phases were used to accomplish this work. Nicotine was extracted out from different tobacco products, by using various chemical solvents, to optimize the better extraction method, this involves the first phase of the study. The hot water extraction method is suitable, because it provides us better nicotine bands followed by the second phase i.e. determination of nicotine by Thin Layer Chromatography. TLC used to obtain retention factor(rf) value of nicotine in different tobacco products. The third phase involves the confirmatory examination of nicotine content in different tobacco products, based on their absorbance radiance in colorimeter followed by the Gas Chromatography analysis. Based on the preliminary examination until yet, nicotine concentration is more in smoking tobacco products (cigarettes) of Indian brands than the International brands.

INTRODUCTION

Plants produce various types of organic compounds i.e. secondary metabolites, among which, alkaloid is an important compound. Alkaloid-containing plants are used for the preparation of medicine, recreational drugs, and poisons. The Solanaceae family (Nightshade) has several species producing alkaloids such as the Nicotiana genus (tobacco plant) and the Solanum genus having 3,000 species to date, which consist of major alkaloids like nicotine, nornicotine, anatabine, and anabasine [17].

Nicotine {3- [2- (N-methyl pyrrolidinone) pyridine]} is a pale-yellow, hygroscopic compound which on exposure with air and light, turns brown having an odor. It is water-miscible in its neutral-amine-base form and decomposes between 60°C to 247°C having vapor pressure 20°C:0. 006kPa. Although, it is presumed that nicotine is served as a natural defense for fungi, bacteria, insects, etc. [21]. Thus, in some countries nicotine is used as an insecticide, which contaminates the food materials [21]. Chemically, Nicotine is made up of two rings, a pyridine ring, and a pyrrolidine ring. The pyrrolidine ring derived from the polyamine putnisite metabolism gradually converted to N-methylpyrrolidone. Pyridine rings originate from the nicotinic acid. Derivatives of nicotinic acid condensed and N-methylpyrrolidinium forms nicotine [22].

Nicotine alkaloid is mainly found in the roots of the Nightshade plants and by the help of various transporters xylem transport nicotine from roots to the leaves. Nicotiana tabacum has various transporters used to transport nicotine to aerial plants such as as-Methyl jasmonate (MeJA), Jasmonate inducible alkaloid trasporter2(JAT2), Nicotine uptake permease (NUP1) and Plasma-membrane transporter. The functioning of each transporter is mentioned as follow:

1.MeJA: when any herbivore attacks the plants, it increases the xylem concentration in leaves, and MeJA signals help to transport nicotine from the site of a plant to the organs of herbivore for the protection of plants.

2. JAT2: belongs to the family of multidrug and toxic compound extrusion (MATE), which is used to transport secondary metabolites, iron, and plant hormone signals. JAT2 is found in the leaves, which restrict tonoplast and help to distribute nicotine into leaf vacuoles.

MATE has further divided into the following categories of transporters such as MATE1, MATE2, JAT1, and PUP (purine permease). MATE1 and MATE2 helps to transport nicotine in roots. NUP1 transports nicotine to root tips where the production of compound molecules is highly active. JAT1 is mostly found in root, stem, and leaves, which indicate nicotine concentration in green leaves. The

plasma membrane-localized transporter transfers nicotine into various cells. Nicotine is also present in the flowering stage, which affects the seed production. MATE1 and MATE2 present in overall flowers. JAT1 present at the time of flower development and also in petal, pistil, and stamen of fully developed flowers. Whereas a small amount of JAT2 is present in the pistil [22].

Majorly Nicotiana rustica and Nicotiana tabacum are used for the manufacturing of tobacco products. Tobacco leaves have approximately 1.5% of nicotine by weight. Tobacco products have been divided into three categories such as smoking, chewing, and sniffing products. Smoking is sub-classified into cigarettes, bidis, cigars, and pipes. Whereas chewing is sub-classified into pan masalas. Cigarette tobacco has approximately 8.0milligram(mg) of nicotine content but at the time of smoking on an average 1-1.5mg is absorbed by the body. Pipe tobacco and oral snuff have the same nicotine concentration as cigarettes, whereas chewing and cigar have half nicotine concentration. Medically, various medical products such as chewing gums, nasal sprays, and nicotine-impregnated patches also have less nicotine content used by an addictive person to quit smoking and sniffing [19].

Effect of Nicotine on Human Body

In the human body, nicotine gets absorbed by various pathways such as skin, lungs, oral cavity, gastrointestinal tract, and urinary bladder. Absorption through the biological membrane is dependent upon its pH. Respiratory system absorbs nicotine 60%-80%. On inhalation, nicotine is absorbed by alveoli of lungs since it has a large surface area for the absorption of tobacco smoke and approximately 1.0mg (range 0.3-2.0 mg) of nicotine is absorbed. The stomach absorbs nicotine poorly due to the presence of gastric fluid. Small intestine easily absorbs nicotine because of large surface area and more alkaline pH. Skin absorbs nicotine base when coming in contact with the tobacco leaves [16,17,18].

In the human body,70%-80% of nicotine is metabolized in the form of cotinine by C-oxidation. The conversion of nicotine into cotinine involves the hydroxylation of nicotine by cytochrome, converting the alkaloid with the help of cytosolic enzyme ultimately producing cotinine. CYP2A6 is the enzyme used by the C-oxidation for the conversion of nicotine into cotinine. Nicotine is easily distributed in the spleen, liver, lungs, and brain whereas adipose tissue`s affinity is comparatively very low [16,17,18].

The human body excretes out nicotine through urine, bile, feces, saliva, gastric juice, sweat, and breast fluid. With a half-life of 2-3 hours(h) nicotine is eliminated from blood. Traces of nicotine and cotinine are also found in the urine of infants when mothers frequently smoke tobacco. The excretion rate of nicotine depends upon the consumption rate of nicotine and also on the pH of urine because when the

pH of urine is more alkaline, the nicotine is reabsorbed by the body and excrete out less nicotine [16,17,18].

Nicotine exposure is estimated by using various biological markers. Commonly used markers are nicotine and cotinine in urine, saliva, hair, and plasma. Urine cotinine is a widely used marker for nicotine exposure. Nicotine and Cotinine have disadvantages as it indicates recent exposure of nicotine in body fluids due to its rapid elimination. Cotinine has 15hrs of half-life in body fluid whereas nicotine has 2hrs of half-life. Hair shows long term exposure to nicotine. Nicotine concentration in plasma primarily depends upon the type of tobacco smoke. Non-smokers plasma has approximately 2.5-8.0ng/ml (nanograms per milliliter) of nicotine concentration whereas smokers have 30-40ng/ml nicotine concentration in plasma [16,17,18].

The intravenous injection of nicotine increases the concentration in blood and brain which produces low dose toxicity while Oral or intraperitoneal produces high dose toxicity. In humans, the lethal dose is approximately 40mg-60mg(milligram) or less than 5mgkg-1[milligram of something per kilogram] (less than 7 drops). Nicotine has various adverse effects on the human body which generate acute and chronic toxicity. Acute toxic effects produce nausea, vomiting, abdominal pain, diarrhea, headache, sweating. More severe effects produce dizziness, weakness, and confusion, progressing to convulsion, hypertension, and coma. Death occurs due to paralysis of respiratory muscle or central respiratory failure. Skin exposure to nicotine leads to severe poisoning. Chronic (long term) toxicity affects various biological systems of the human body like the reproductive system, immunological followed by genetic and carcinogenic effects, cardiovascular diseases, and gastrointestinal effects [16,17,18].

Common vegetables and plants mostly from the Solanaceae (Nightshade) family, shown to have very low amount of nicotine. E.g.: (1) Tomato has an average of 7.1-7.3 ng/g wet weight which means 7.1 ng of nicotine in every 1g of tomato. (2) Potatoes also contain nicotine alkaloid called Solanine, which is highly concentrated on the skin. (3) Eggplant as a concentration of 100ng/g of nicotine which means 10kg of eggplant have similar nicotine content as a stick of cigarette. (4) Tea (green and black) contains a small amount of nicotine.

The purpose of this study to estimate nicotine content in tobacco products by TLC examination followed by the colorimetric and gas chromatographic examination.

Literature Review

In India various forms of tobacco are consumed by people such as smoking (cigarettes and bidis), pan masalas (chewing tobacco), etc., causing severe health problems. For public interest, the ingredients

of smokeless and smoking tobacco are examined by S Reddy, Sujatha.et al in their study. Estimated nicotine in smoking tobacco (cigarettes and bidis) and in pan masalas (chewing tobaccos). They analyzed, relatively high nicotine concentration in tobacco from bidis(26.9mg/gm) as compared to cigarettes(15mg/gm) and chewing tobacco has 3.4mg/gm nicotine concentration. Based on their study, Indian brands have more nicotine concentration than International brands. Bidis have more nicotine than cigarettes whereas chewing tobacco has less nicotine than smoking tobacco [2].

In the study by HOSU, Anamoria. et al; twelve popular brands of cigarettes were examined by the TLC for evaluation of nicotine concentration. Nicotine bands were observed under UV light at 245nm and in visible light, after Dragondorf`s reagent used to visualize nicotine bands. Nicotine showed orange color bands in a yellow background. The result showed significant variation in nicotine concentration, and counterfeiting was checked by comparing printed concentration [3].

Hubble-Bubble (HB) smoking is in tradition and known under various names such as oriented pipe, water pipe, Arghileh, Narghileh, Hookah, Seesha, etc. HB smoking is liked by every age group person but mostly, by young teenagers and women. In the study by, Haididi, KA. et al; cigarettes tobacco was compared with HB smoking tobacco for nicotine content estimation. They observed unflavored HB tobacco have (713 mg/head) nicotine and flavored tobacco have (67mg/head) nicotine content. 70 cigarettes equivalent to one head of unflavored tobacco. Flavored HB tobacco contains on average one third nicotine equivalent to 20 cigarettes. Based on the study, it was concluded that HB smokers are not at lesser risk than cigarette smokers concerning nicotine dependence [4].

Electronic cigarettes (EC) are the alternative to smoking, these are battery-powered devices that produce vaporized nicotine solutions. EC vapors generate flavors and similar sensations like inhaled tobacco smoke, but no tobacco smoke or combustion is inhaled. In a study by Goniewicz, ML. et al; examined various EC samples for nicotine content and its effect on the human body. The nicotine content of the same batch varied up by 12% (RSD) and different batches of the same brand, mean difference ranged from 1% to 12% and nicotine concentration in vapor lies in range 2mg to ~15mg, which is less than conventional cigarettes ranges.

In a study by (9) Hegde and Nanukuttan, nicotine concentration of five ST products was estimated. The analysis showed that plain tobacco has more nicotine content (2.22 mg/g) followed by ghutka, khaini, pan masala and supari, respectively. The variation in nicotine concentration of these products

depends on the difference in their manufacturing, size of tobacco cuttings (10). ST products pH is a crucial determinant of nicotine absorption through the nose and mouth. (11) Nicotine is unionized and rapidly absorbed in alkaline pH whereas ionized and does not cross membrane in low acidic pH. (12) The pH of ST products varies from 9.47 to 5.24, which was highest for khaini and lowest for plain tobacco. (10) The difference in pH could be attributed to different manufacturing methods, additives used and moisture contamination.

Nicotine content depends upon the type of smoked and smokeless tobacco product. In the study by Sharma, P et. al: examined 71 smokeless and smoked products for nicotine estimation. Smoked tobacco contains (1.01 to 13.0 mg/rod) nicotine, while smokeless tobacco has (0.8 mg/g to 50.0 mg/g) nicotine concentration. There are various factors which affect the nicotine concentration in tobacco products [13].

Smokeless tobacco has various other forms such as Kaddipudi, mishri, and zarda. Kaddipudi on average contains 5.3mg/g of tobacco. Zarda is added to betel leaves as per the customer taste (strong or light). Zarda contains 20.35mg/g nicotine content. For public health, quantification of nitrosamines and polycyclic aromatic hydrocarbons, disease markers, and of all gaseous toxins of each product (carbon monoxide, tar, and other oxides) was done. To create awareness among people regarding the harmful effects of these products on human health [14,15].

Tobacco has various forms such as sun-cured tobacco, flue-cured tobacco, and air-cured dark tobacco i.e. nicotine content varies in different samples. Oriental tobacco has 1.8-12.6 mg/g nicotine content, whereas Virginia tobacco has 6.52-60.4mg nicotine content and Burley tobacco has 35.6-47.3 mg nicotine concentration. Mostly, flue-cured and air-cured tobacco is used for the manufacturing of cigarettes in many countries like the UK, USA, and India, etc. Predominant types used in Canada, Japan, China, and Australia etc. Today, American blended cigarettes are mostly sold by retailers in many countries [6].

OBJECTIVE:

1. Collection of smoking & chewing type of tobacco products from local and international markets.
2. Extraction of nicotine from all samples.
3. Separation of nicotine by TLC.
4. Confirmatory detection of nicotine by colorimetric methods and gas chromatography.

Material required and Methodologies

Commercial smoking tobacco products cigarettes both Indian and International brands were obtained from the local retail market. Ten cigarettes samples (Five Indian and Five International brands) were

used for the study. Pure nicotine strips dissolved in water, then solution is used as standard of nicotine. Tobacco weight was estimated by measuring each cigarette of both brands. For each brand of cigarettes, tobacco was removed from the wrapping and pooled in the chemical solvent for nicotine extraction. Nicotine presence was estimated by preliminary examination i.e. TLC and quantified by confirmatory test. This study was carried out in three phases as mentioned below.

Phase 1: Extraction of nicotine from smoking (cigarettes) tobacco products
Three different chemical extraction methods were used to extract out nicotine as mentioned below:

1. First Extraction method:
    i. 20mg of tobacco was weighed.
    ii. Add 10ml of 1% KOH () in methanol and grind in a vortex pestle manually.
    iii. Incubate overnight below 45°C.
    iv. Centrifuge for 5 min at 10,000 rpm
    v. Supernatant (top layer) was then used for nicotine analysis.
2. Second Extraction method:
    i. A 5gm tobacco sample was weighed.
    ii. Add 10ml ethanol and water in a ratio of (4:1) and grind in a vortex pestle manually.
    iii. Incubate overnight at 50°C.
    iv. Centrifuge for 5 min at 10,000 rpm
    v. Supernatant (top layer) was then used for nicotine analysis.
3. Third extraction method:
    i. 0.5gm of tobacco was weighed.
    ii. Add 10 ml hot water and grind manually in a vortex pestle.
    iii. Incubate overnight at 50°C.
    iv. Centrifuge for 5 min at 10,000 rpm.
    v. Supernatant (top layer) was then used for nicotine analysis.

The organic layer was used to analyze nicotine content in different cigarettes followed by the second phase of study i.e. Thin Layer Chromatography examination.
Phase2: Nicotine examination by TLC:
The extracted layer or supernatant of each cigarette was examined on the TLC plate to calculate the retention factor (rf) value of nicotine. Following procedure was used for examination:

Step1: Preparation of TLC plate:

i. Three clean glass slides were taken.
ii. In a beaker thin slurry was prepared by adding 3gm of silica powder and 6ml water.
iii. Slurry was poured onto glass slides and dried at room temperature. Glass slides were kept in a hot air oven at 55° for 15-20min, for activation of TLC plates.

Step 2: Preparation of solvent:

i. Solvent was prepared by adding 80%ethanol and 1N HCL v/v in (10:0.4 ratio).

Step3: Loading of TLC plates:

i. With the help of a tip, point was marked and 10-15µl (microliter) extracted solution was loaded.
ii. Vertically glass plates were placed into solvents and covered with a lid.

Step4: Visualization/Detection of TLC plates:

i. After completion of running, the TLC plates were dried and examined under UV (ultraviolet) light at 245nm (nanometer) for nicotine bands.

Step5: Calculation of retention factor(rf) value:

Rf value was calculated by measuring the distance travelled by solute and solvent on a chromatographic plate.

Result and Discussion

The weight of Indian brand cigarettes on an average is 1.04mg (Table1). The average weight of tobacco in Indian brand cigarettes is 0.67mg (Table1). The weight of International brand cigarettes on average is0.65mg (Table1). The average weight of tobacco in International brand cigarettes is0.45mg (Table1).

S.No.	Cigarette's Name	Cigarettes weight(mg)	Tobacco weight(mg)
	Indian Brand's		
1.	Classics	0.93	0.60
2.	Marlboro advance	0.92	0.59
3.	Gold Flake	0.99	0.61
4.	Gudang Garam	1.33	1.12
5.			
	Overall average:	1.04	0.67
	International Brand's		
1.	Pine	0.52	0.34
2.	Esse Lite	0.51	0.35
3.(a)	Mond-Green Apple	0.55	0.36
(b)	Mond-Strawberry	0.54	0.36
(c)	Mond-Cherry	0.53	0.37
4.	Black	0.93	0.67
5.	Win	0.97	0.73
	Overall average	**0.65**	**0.45**

Table 1: Indian and International Brands smoking tobacco (cigarettes) samples.

Indian brand five out four and International brand five out three samples were analyzed by the TLC and chromatograms were observed under UV light followed by rf value calculation as mentioned Table2, compared with nicotine standard rf value Table3.

Nicotine standard

Fig 1: Nicotine standard

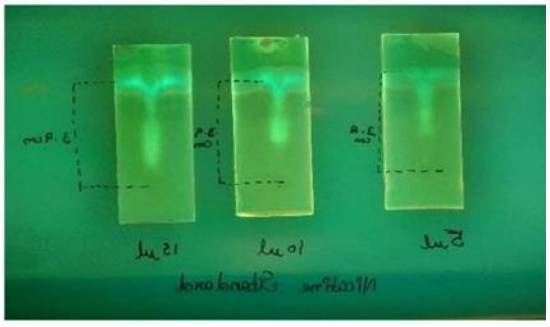


Fig 2: Nicotine chromatograms observed under UV light.

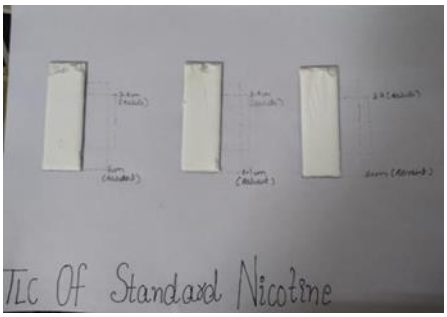


Fig 3: Nicotine standard TLC examination having different rf values(i)0.63, (ii) 0.65, (iii)0.65.

Indian brand smoking tobacco (cigarettes)

Fig 4: Indian brand sample1 (Classic) and International brands sample1 (Pine).

1. Sample1 (Classic)

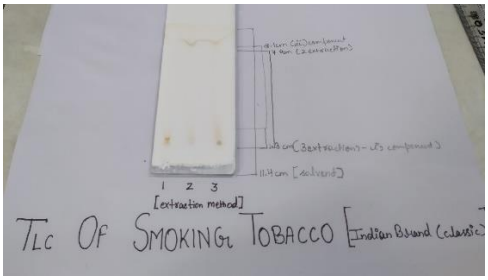


Fig 5: TLC of classic sample.

Fig 6: centrifuged sample of Classic.

[Three extraction methods were used to extract nicotine, to optimize better extraction method. The concentration of loaded samples was 15µl. The third extraction method provided dark nicotine bands, so the sample was again run as shown in Fig.7. Calculated rf values mentioned in Table2.]

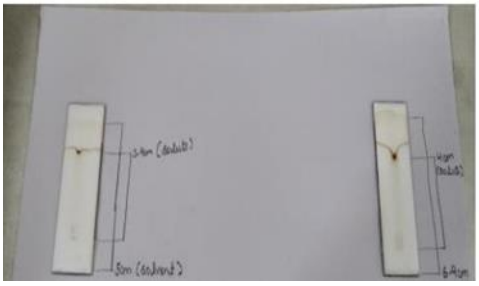


Fig. 7: Bright bands observed by third extraction method

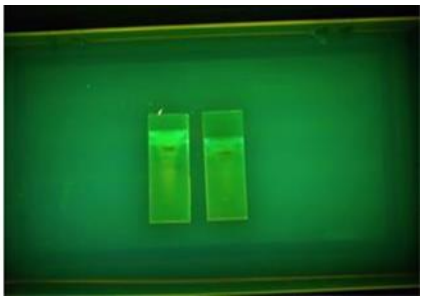


Fig. 8: Chromatograms observed.

[Loaded sample extracted by third extraction procedure having concentration 15µl. Distance covered by solvent as plate1-5cm and plate2-6.9cm, whereas loaded sample distance from baseline in plate1-3.9cm, and plate2-4cm, having rf value plate1=0.78cm, plate2=0.57cm.]

2. Sample 2 (Marlboro advance)

Fig. 9: Marlboro advance cigarette (smoking tobacco)

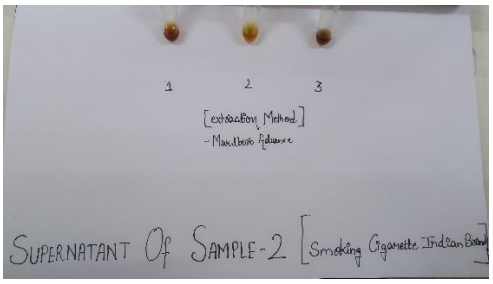


Fig. 10: Centrifuged sample.

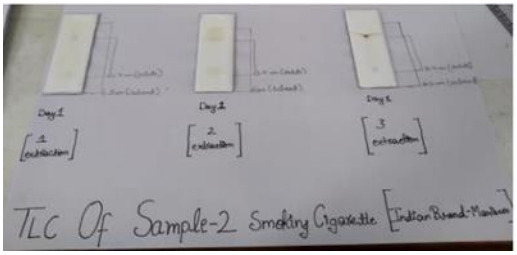


Fig. 11: Nicotine extraction (TLC).

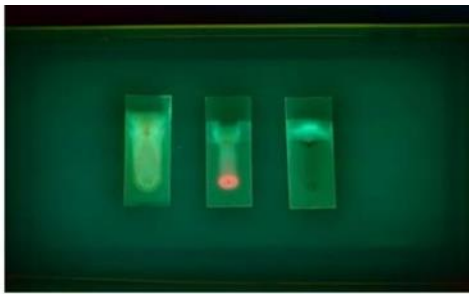
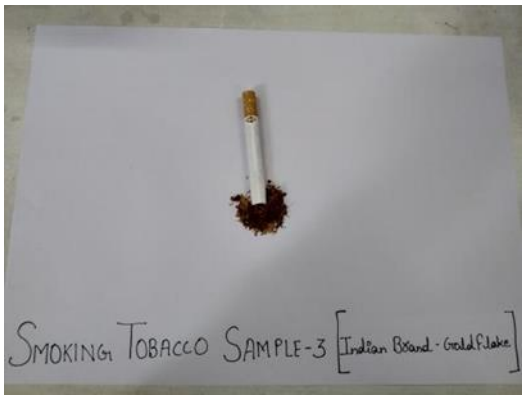


Fig.12: Nicotine chromatograms observed Under UV light.

[Three extraction methods were used to extract nicotine. The concentration of the loaded sample was 15µl on each plate. Distance covered by solvent in plate1=6.5cm, plate2=6cm, plate3=6.2cm, whereas loaded sample distance from baseline for plate1=3.7cm, plate2=3.4cm, plate3=3.4cm. As calculated rf values mentioned in Table2.]

3. Sample 3 (gold flake)



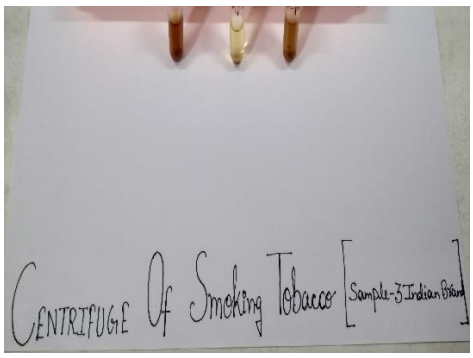
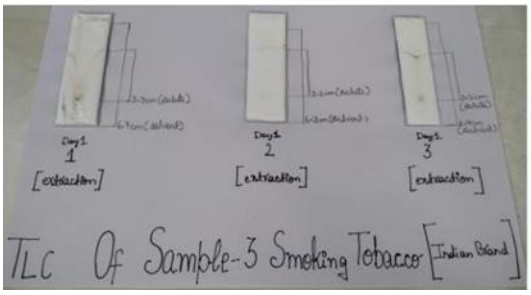


Fig. 13: Gold flake cigarette (Smoking tobacco). Fig.14: Centrifuged sample.



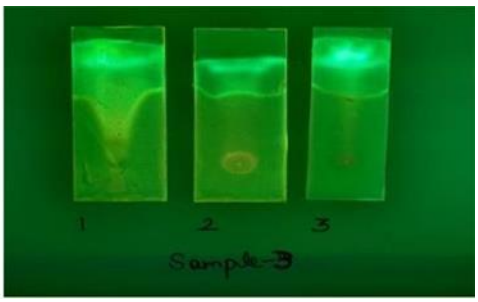
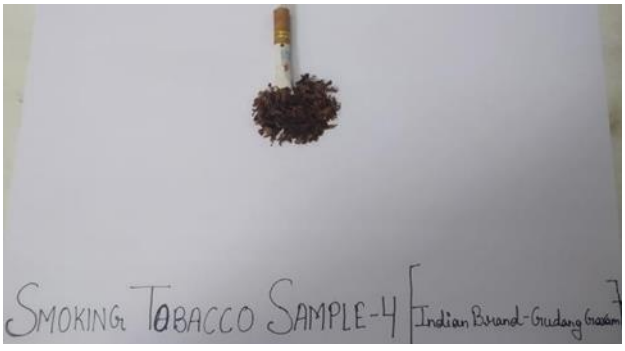


Fig. 15: Nicotine extraction (TLC) Fig.16:Chromatograms were observed under UV light.

[Three extraction methods were used to extract nicotine. The concentration of the loaded sample was 15µl on each plate. Distance covered by solvent in plate1=6.7cm, plate2=6.3cm, plate3=6.9cm, whereas loaded sample distance from baseline for plate1=3.3cm, plate2=3.2cm, plate3=3.2cm. As rf values mentioned in Table2.]

4. Sample 4(Gudang garam)



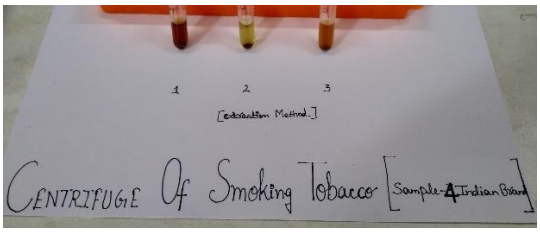


Fig.17: Gudang garam cigarette(Smoking Tobacco) Fig. 18:Centrifuged sample.

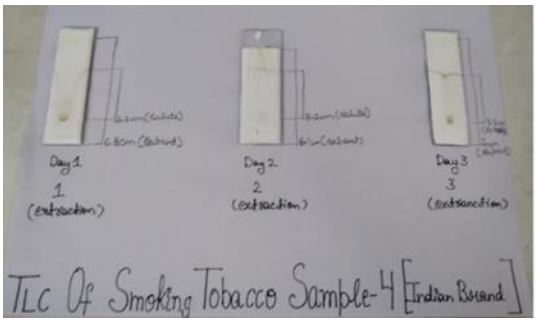


Fig. 19: Nicotine Extraction (TLC).

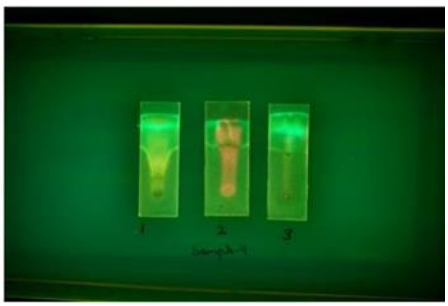


Fig. 20: Chromatograms observed under UV light.

[Three extraction methods were used to extract nicotine. The concentration of the loaded sample was 15µl on each plate. Distance covered by solvent in plate1=6.8cm, plate2=6.1cm, plate3=7cm, whereas loaded sample distance from baseline for plate1=3.2cm, plate2=3.2, plate3=3.2cm, rf values mentioned in Table2.]

International brand smoking tobacco (cigarettes)

Fig. 21: International brand smoking tobacco cigarettes sample2: Esselite, Sample3: Mond having sub-groups(a) green apple, (b) strawberry, (c) cherry. Sample4: Black, Sample5: Win.

1. Sample 1:(Pine)

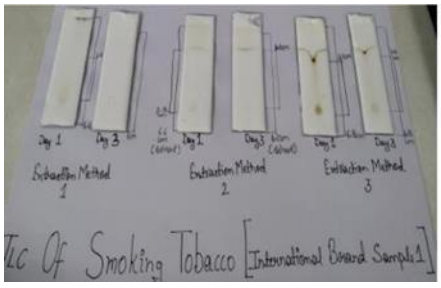


Fig. 22: Nicotine Extraction (TLC).

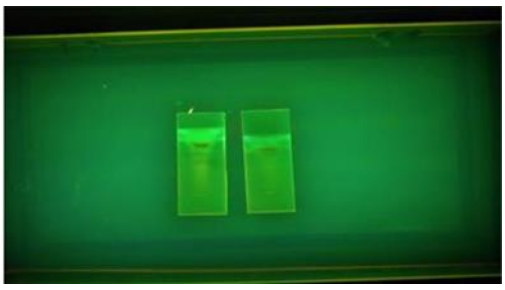


Fig. 23: Chromatograms were observed under UV light.

[Three extraction methods were used to extract nicotine. The concentration of the loaded sample was 15µl on each plate. Distance covered by solvent in plate1(a)=6.6cm, plate1(b)=6cm, plate2(a)=6.6cm, plate2(b)=6.1cm, plate3(a)=6.8cm, plate3(b)=6.8cm ,whereas loaded sample distance from baseline for plate1(a)=2.6cm, plate1(b)=not visible, plate2(a)=2.8cm, plate2(b)=2.6cm, plate3(a)=2.3cm, plate3(b)=2.1cm. Rf values mentioned in Table2.]

2. Sample2:(Esse light)

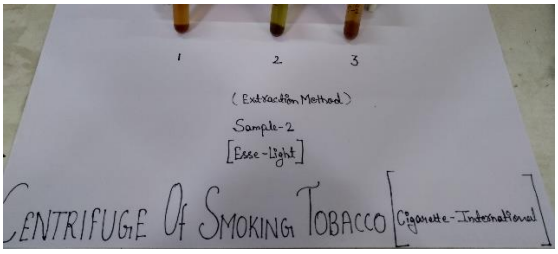


Fig. 24: Centrifuged sample.

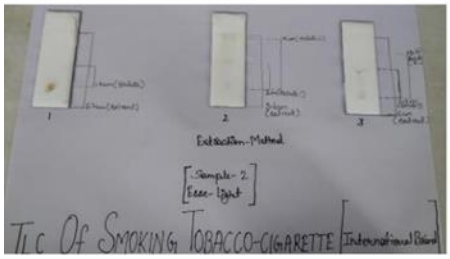


Fig. 25: Nicotine extraction (TLC).

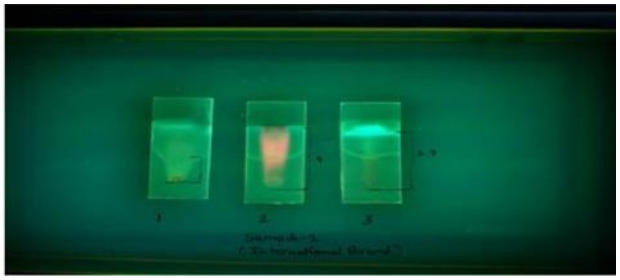


Fig. 26: Chromatograms were observed under UV light.

[Three extraction methods were used to extract nicotine. The concentration of the loaded sample was 15µl on each plate. Distance covered by solvent plate1=5.7cm, plate2=5.6cm, plate3=6cm, whereas loaded sample distance from baseline for plate1=1.cm, plate2=4cm, plate3=3.7cm. Rf values mentioned in Table2.]

3.Sample3(a): Mond- Green apple

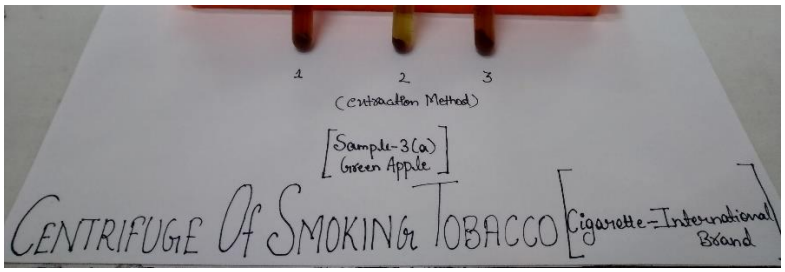


Fig. 27: Centrifuged sample.

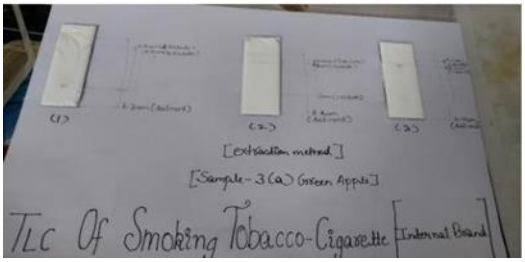


Fig. 28: Nicotine Extraction (TLC)

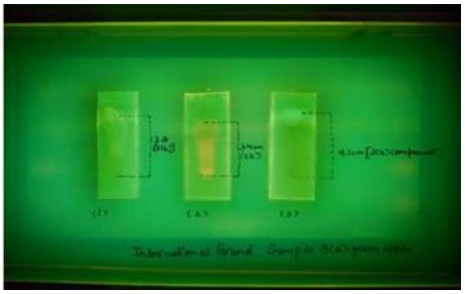


Fig. 29: Chromatograms were observed under UV light.

[Three extraction methods were used to extract nicotine. The concentration of the loaded sample was 15µl on each plate. Distance covered by solvent in plate1=6.3cm, plate2=5.8cm, plate3=8.4cm, whereas loaded sample distance from baseline for plate1=3.9cm, plate2=3.4cm plate3=4.1cm. Rf values mentioned in Table2.]

Cigarettes Names	Retention Factor (rf) Value Extraction Method		
Indian Brands	*First method*	Second method	Third method
1.Classis	0.62	0.63	0.59
2.Marlboro advance	0.56	0.56	0.56
3.Gold Flake	0.49	0.50	0.46
4.Gudang Garam	0.47	0.52	0.43
5. Not run yet	-	-	-
International Brands	*First method*	Second method	Third method
1.Pine	0.39	0.42	0.33
	Not visible	0.39	0.30
2.Esse Lite	0.12	0.37	0.36
3.(a)Mond -Green apple	0.61	0.58	0.64
(b) Strawberry	-	-	-
(c) cherry	-	Not Run Yet	-
4.Black	-	-	-
5.Win	-	-	-

Table 2: Indian and International brand cigarettes nicotine rf values extracted out by three different methods. These values need further validation.

S.No.	Concentration Of solute	Retention Factor Rf value
1.	First 10µl	0.63
2.	Second 15µl	0.65
3.	Third 20µl	0.65

Table 3: Nicotine standard rf value obtained by loading different sample amount.

Tobacco abuse is a worldwide health problem, like drug and alcohol abuse. In India, it is estimated that for people above 15 years of age, approximately 57% of the population uses tobacco in many

different forms. Mostly, people smoke Bidis, approximately 12% smoke cigarettes, and others use chewing tobacco. In India, 86% of tobacco is used for making smoking products, 12% for smokeless products, and renaming used for domestic purposes [1].

In our study, nicotine bands show fluorescence in UV light at 245nm. Different samples have different intensity, to separate out nicotine bands on chromatographic plates, indicating that concentration of these compounds depends on the type of cigarette brands or samples. Indian brands sample 1 and2 shows more fluorescence than other samples, some samples indicate the same level of nicotine concentration. International brand cigarettes show fluctuation in their rf values so, the study needs further validations. The International brand sample 1, extracted solution of two different days (day1 and 2) were run on the TLC plate to check the difference in nicotine concentration, but day 1 provides more rf value. nicotine concentration in cigarettes differs because of different brands and manufacturing procedures. There are various factors, which make Indian cigarettes different from International brands cigarettes like plant variety, cultivation, curing method, wrapping design and presence or absence of a filter, etc. These factors are also useful to identify the toxic level of chemicals in tobacco and tobacco smoke.

CONCLUSION

This study concluded that Hot water extraction method (third method) indicates more bright nicotine bands. Indian brand cigarettes have more nicotine concentration than International brand cigarettes. TLC is a simple and low-cost technique for the evaluation of nicotine from various samples like tobacco products, urine, and other biological samples. It helps to identify smokers and non-smokers, after examining other samples. This study needs further validation.

Considering findings of the present study and other published data, it is suggested that the nicotine concentration decreases as low as possible in tobacco products to reduce the harmful effect of long-term nicotine addiction. Although avoiding or quitting smoking is the beat, safe and effective way to minimize health problem.

Future Scope

Tobacco products such as smoking (cigarettes, bidis, and hookah, etc.), sniffing and chewing (pan masalas), nicotine content estimated by using different analytical techniques, can be compared to analyze how much the amount differs in their result. There is a need to identify new diseases and their harmful effect on the human body due to nicotine for future studies.

Such a study of Nicotine concentration would help in understanding of the pharmacology of nicotine and how nicotine produces addiction and influences smoking behaviour provides a necessary basis for therapeutic advances in smoking cessation intervention.

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