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GCMS Analysis of Chemical Stimulants and Carcinogens in Tobacco Leaves and Selected Tobacco Products

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Abstract- ***In spite of public awareness of high death rate of tobacco-related cancer worldwide, high prevalence and addiction to tobacco smoking in Nigeria as a major health risk, is due to poor and weak tobacco control law implementation. The presence of addictive nicotine alkaloid, and carcinogenic inorganic metals, and organic tobacco-specific nitrosamine and polycyclic aromatic hydrocarbons are the toxic load that shows high positive correlation with various known cancer types. Identification, quantification and characterization of nicotine alkaloid, and carcinogenic PAHs, TSNA and heavy metals in seven brands of tobacco cigarettes (TS1-TS7) consumed in Nigeria and raw tobacco leaves (TS8) used as baseline were determined using GC-MS. Mass spectrum fragmentation ladder, base peaks, and diagnostic m/z ions rules were part of the rules used for structural deduction and validation where library hit percentage was below 50 confidence level. TS1 brand revealed 1.35% – 41.78% of tobacco wax, TS2 cigarette showed 1.93% – 31.23% concentration of TSNAs), a diverse profile of 1.22% – 25.11% was found in TS3, High levels of Fatty Acid Amides and Esters were recorded in TS4: 1.68% – 34.44%, TS5: 2.05% – 33.17%, TS6: 1.36% – 22.50%), TS7: 4.79% – 32.53%, and TS8 tobacco leaves showed remarkable high tobacco wax percentage range of 1.75% – 45.41%. TSNA (tobacco-specific nitrosamines) ranges from 7.40% (TS8) to 34.46% (TS4), with NNK-TMS derivatives also present (2.91-17.00%). Tobacco wax content varies significantly (9.29-41.07%), while phytosterol is prominent in TS7 (41.71%). Monoterpene derivatives, diterpenes, and fatty acid amides are also detected across samples. Carcinogenic PAHs are present in some samples (1.33-3.63%). While TSNA-NNK dominated TS1 to TS8. Spearman correlation matrix analysis of (r and p values) for the Qion analysis indicated TS5 and TS6 have the highest correlation among cigarette brands at 0.998, suggesting nearly identical manufacturing or environmental carcinogen signatures. The GC-MS structural deduced and validated chemical compounds showed TS4-TS5 with a ($r = 0.914$, $p < 0.01$), TS4-TS2 ($r = 0.986$, $p < 0.01$) and TS1-TS7 ($r = 0.906$, $p < 0.01$) across TS1 to TS8. TS2 brand has the highest toxin load, followed by TS3 (43%) and TS4 (41.68 %). While the PAH concentration was highest in TS6 and TS3 with 3.63% and 2.56% load respectively. Due to the high reactivity of carcinogen, a complex of NNK-TMS was detected eluting with tobacco waxes, phytosterol and tocopherol as well, as a perfect trap to mask carcinogen in all samples. The structural deduction rule revealed that the notable nicotine alkaloid in tobacco is the source of most NNK and related TSNA carcinogen in tobacco cigarettes which was also found along other alkaloid in TS8. The toxicological profile in all TS1-8 revealed heavy loads of toxins, trace PAHs, heavy waxes.***

Keywords: Tobacco cigarette and leaves, Carcinogen, Polycyclic Aromatic Hydrocarbon, tobacco-specific nitrosamine, and GC-MS.

I. INTRODUCTION

Numerous chemicals found in tobacco cigarette smoke are directly associated with cancer prevalence and mortality. In spite of global awareness and significant public health risk linked with tobacco use, persistent use of tobacco products daily in Nigeria is a major health problem, among low income earners and the younger generation. Tobacco industry with major both local and foreign manufacturers located majorly in Northern Nigeria and other parts of the nation indicate weak enforcement of tobacco control laws and regulations, resulting in questionable safety, assurance and quality of tobacco products. The research aims in resolving tobacco matrix problem and the knowledge gap for a forensic chemical map peculiar to selected popular tobacco products m anufactured and consumed in Nigeria. The key findings of the research will have significant consequences for public health policy and regulation in the tobacco industry in Nigeria.

Although public awareness of these harmful carcinogens and their health impact on smokers, users find it extremely difficult to withdraw from tobacco-related smoking due to nicotine addiction. The major alkaloid in the tobacco plant – nicotine when present in smoker's lungs easily crosses the brain-lipid membrane reaching the brain within seconds, eliciting a relaxed calm mood and deepens nicotine dependence on tobacco products. This effect leads more to users' addiction and possible high cancer prevalence [2]. Worldwide, tobacco smoking is a leading cause of death, with over 18 million new reported cases of tobacco-related smoking [3]. Consequently, tobacco smokers are prone to different multiple cancers, including mouth, bladder, lung, throat, esophagus, stomach and cervical cancers.

Complex mixture of chemicals in tobacco products requires a thorough analysis of individual chemical classes and combined toxic effects. Tobacco health risk is attributed to the interaction of different components. A combination of heavy metals, TSNA, volatile organic compounds, polycyclic aromatic hydrocarbons in commercial tobacco brands can form complex chemical adducts and disrupt normal metabolic processes. A forensic and toxicology assessment study examining various factors such as tobacco soil chemistry, tobacco additives, tobacco curing, and manufacturing processes can help shape the forensic chemical map of tobacco products in Nigeria. To assess the potential health risks associated with tobacco smoking, this study uses modern spectroscopic and chromatographic techniques to identify and determine the chemical composition of these tobacco products and assess the potential health risks associated with tobacco smoking.

II. STATEMENT OF PROBLEM

Tobacco leaves and products are known for health risk load consisting of known carcinogens, including Polycyclic Aromatic Hydrocarbons, phenolic volatile organic compounds, Group 1 and 2A carcinogenic metals such as arsenic, cadmium, copper, chromium, nickel and lead with neurotoxic effects. They also contain psychoactive and additive alkaloids, as well as notorious types of tobacco-specific nitrosamines, specifically *N*-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), along with their derivatives.

Aim and objectives of study

This research focuses on the extraction, identification, and quantification of carcinogenic chemicals and stimulants in tobacco products and leaves using ICP-OES and GC-MS analysis. The objective is to estimate chemical stimulants per cigarette brand and compare the chemical composition between tobacco leaves and selected brands in Nigeria.

2.1 Cancer Prevalence

Tobacco remains a leading cause of preventable death worldwide, with over 18 million new cancer cases diagnosed globally in 2018 [3]. Extensive epidemiological evidence has linked tobacco smoking to various malignancies, including lung, oral cavity, throat, esophagus, stomach, pancreas, kidney, bladder, and cervical cancers [12]. The number of smokers has reduced worldwide from 51.1 to 21.6%, and 33.3 to 16.5% among men and women respectively between 1990 and 2019. However, there is high prevalence of smoking-related cancers due to the long latency of cancer development and bioaccumulation of harmful carcinogens in the body [10]

2.2 Carcinogens and Stimulants in Tobacco (TNSAs, PAHs, Nicotine and Heavy metals)

PAHs in Tobacco

Polycyclic aromatic hydrocarbons (PAHs) are commonly classified into three main categories; pyrogenic, petrogenic, or biogenic forms. These compounds are believed to be extremely hazardous organic pollutant, strongly hydrophobic, thermally stable and known to cause cancer. Several well-known PAHs occur in tobacco including phenanthrene ($C_{14}H_{10}$), benzo[a]pyrene ($C_{20}H_{12}$), benzo [a]anthracene ($C_{18}H_{12}$) chrysene ($C_{18}H_{12}$), and naphthalene. Structurally, PAHs consist of two or more aromatic rings that are fused together. Their relatively high degree of unsaturation (high DBE) contributes to their chemical stability and makes them resistant to breakdown in the environment.

Health Impact of PAHs: Concentration of PAHs in different tobacco brands is based on the tobacco type, curing methods, and manufacturing processes [4]. However, increased asthma, higher cardiovascular

attacks, and obstructive lung cancer are observed and directly associated with prolong exposure to PAHs. According to a study by Adesina (2021), PAH levels in smokers' lungs were estimated to range from 956.1 to 1607.2 ng/g, resulting in increased risk of respiratory and cardiovascular attacks [1].

2.3 Tobacco-Specific Nitrosamines (TSNAs)

N-nitrosonornicotine (NNN), and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), are the major nitrosamines found in tobacco products and smokers' saliva. When NNN or NNK is absorbed in the liver and metabolically activated by cytochrome p450 enzymes, it produces DNA adducts, resulting in mutation, carcinogenesis and tumor growth. Beyond the curing stage, recent research indicates that the continued accumulation of DNA adducts from TSNA exposure is a primary driver in the silencing of tumor suppressor genes, specifically through the methylation of the p53 pathway in chronic users [6].

2.4 Health Effects of Tobacco Smoking

Immediate effects include the formation of free radicals, depletion of circulating antioxidants (Vitamin C and Provitamin A), and increased systemic inflammation. Smoking is also a cause of periodontitis, acute myeloid leukemia, and malignancies across multiple organs. In pregnant smokers, it can lead to miscarriage or fetal underdevelopment. Furthermore, smoking significantly modifies the lipid profile by lowering high-density lipoprotein (HDL) and increasing serum cholesterol, triglycerides, and low-density lipoprotein (LDL). Current clinical observations further confirm that the metabolic disruption caused by smoking extends to systemic insulin resistance and significant elevations in pro-inflammatory biomarkers, which accelerate the onset of coronary artery disease [5].

2.5 Alkaloids and Heavy Metals

Nicotine is the primary stimulant in tobacco with high addictive potential; it reaches the brain in less than seven seconds after inhalation, inducing a temporary state of calmness. Tobacco plants are also efficient at bioconcentrating heavy metals from the soil. This study focuses on highly carcinogenic metals such as arsenic (As), cadmium (Cd), chromium (Cr), and nickel (Ni), alongside the neurotoxic metal lead (Pb). The combined toxic load of heavy metals, nicotine alkaloid and TSNA accounts for the toxicological hazard profile of tobacco brands and consequent multiple organ toxicity and carcinogenic effects in smokers [9].

III. MATERIALS AND METHOD

3.1 Sample Collection and Preparation

Tobacco cigarette samples (TS1–TS7) and pure dried tobacco leaves (TS8) were obtained from the Ado-Odo/Ota LGA in Ogun State. Tobacco leaves sample was identified by a botanist as *Nicotiana tabacum L*. which belong the Solanaceae family, with herbarium number LUH:100789. The samples were prepared extraction and structural analysis. Liquid extractions were performed using high-purity solvents, and silylation was employed where necessary to enhance the volatility of polar constituents.

3.2 GC–MS Analysis

Materials and Reagents:

Dichloromethane (DCM), n-hexane, anhydrous sodium sulfate, and a calibration standard containing 24 polycyclic aromatic hydrocarbons (PAHs).

Analytical Procedure: One-gram (1 g) of dried, homogenized tobacco sample was extracted with a DCM: hexane (1:1) mixture via sonication for several minutes. The extract was filtered through anhydrous sodium sulfate and concentrated using a rotary evaporator. The purified extract was then injected into the GC–MS system. GC–MS peaks were identified through mass spectral library matching, retention indices, and de novo structural deduction.

PAHs Targeted Ion Analysis: Targeted quantification of 24 PAHs was performed for samples TS1 through TS8. The method utilized specific quantitation ions (QIon) for target compounds across all 24 standard PAHs.

3.3 Structural Analysis and Validation

A Gas Chromatography-Mass Spectrometry (GC–MS) equipped with an ALS Vial autosampler was used to generate mass spectra for TS1 – 8 samples. Further identification was performed using the NIST

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2023 Library (NIST Standard Reference Database 1A).

De Novo Structural Deduction Rules: Where library hit failed, a manual De novo structural interpretation and validation was employed based on the following chemical rules: a) True molecular ion identification, b) base peak diagnostic matching, c) Mass Spectra fragmentation ladder, d) Leaving group, e) Chemical class identification, f) Carbon count, g) Functional groups h) Molecular formula i) DBE and Molecular weight calculation, j) Leaving group bond connectivity for chemical plausible structural rebuild, k) Nitrogen Rule for odd and even $M+$. M^+ cores their corresponding substituents, diagnostic ion references. Key cleavages (including alpha, beta-cleavages and McLafferty rearrangement), and Predicted Fragments.

3.5 Statistical Analysis

Statistical analysis was evaluated using Spearman correlation matrix (r and p). Relative abundances of GC–MS peaks were calculated based on percentage maximum intensity to normalize data across the diverse tobacco matrices using IBM SPSS 22.

IV. RESULTS AND DISCUSSION

4.1 GC-MS Quantification of Priority PAHs

The organic fraction of the tobacco samples was analyzed for 24 priority polycyclic aromatic hydrocarbons (PAHs). Table 4.1 summarizes the quantified concentrations (ppb) for the most significant carcinogenic markers identified across the eight samples.

Table 4.1: GC-MS Quantification of Priority PAHs in TS1–TS8

Target PAH	TS1	TS2	TS3	TS4	TS5	TS6	TS7	TS8	IARC Class
Naphthalene	8.21	8.21	8.21	8.21	8.21	8.21	8.21	8.21	2B
Phenanthrene	15.41	15.37	15.40	15.38	15.45	15.41	15.43	15.36	3
Fluorene	16.23	16.22	16.23	16.22	16.22	16.25	16.22	16.22	3
Acenaphthene	12.12	12.12	12.11	12.11	12.13	12.11	12.13	12.14	3
Chrysene	0.27	3.38	4.67	2.18	6.20	3.90	3.57	2.71	1
Benz[a]anthracene	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.00	1
Benzo[a]pyrene	1.11	1.05	0.88	0.92	0.90	0.84	0.99	0.80	1
Benzo[e]pyrene	0.31	0.33	0.32	0.32	0.32	0.35	0.37	0.36	3
Dibenz[a,h]anthracene	0.02	Trace	0.12	0.64	Trace	0.02	0.11	Trace	2A
1-Methylpyrene	1.56	1.57	1.66	1.95	1.61	1.27	1.54	2.41	3
Fluoranthene	0.08	0.06	0.02	0.04	0.08	0.07	0.06	0.18	3

2-Phenyl-1H-benz[f]indene	0.59	0.82	1.15	0.49	1.28	0.96	2.36	0.55	3

Note: Quantified in ppb (µg/L). "Trace" indicates detections confirmed via QIon but below the lowest calibration limit.

Spearman Correlation Matrix (rs)

	TS1	TS2	TS3	TS4	TS5	TS6	TS7	TS8
TS1	1.000	0.930	0.916	0.874	0.921	0.923	0.902	0.930
TS2	0.930	1.000	0.986	0.958	0.991	0.993	0.972	1.000
TS3	0.916	0.986	1.000	0.965	0.988	0.993	0.993	0.986
TS4	0.874	0.958	0.965	1.000	0.932	0.944	0.944	0.958
TS5	0.921	0.991	0.988	0.932	1.000	0.998	0.981	0.991
TS6	0.923	0.993	0.993	0.944	0.998	1.000	0.986	0.993
TS7	0.902	0.972	0.993	0.944	0.981	0.986	1.000	0.972
TS8	0.930	1.000	0.986	0.958	0.991	0.993	0.972	1.000

Samples TS5 (6.20 ppb) and TS3 (4.67 ppb) exhibited the highest levels of Chrysene. TS3 contained high relative abundance of heavy waxes. These lipid matrices likely acted as chemical "traps," effectively sequestering these high-molecular-weight carcinogenic NNK during tobacco curing. Although TS8 is a raw, unburnt sample, it contained 2.71 ppb of Chrysene and 12.14 ppb of Acenaphthene. PAHs like Fluorene and Phenanthrene indicated (15–16 ppb) across all samples. All p-values are < 0.01 showing significant correlations among TS1 to TS8 irrespective of whether it is pure tobacco leave sample or processed cigarette brand. TS5 and TS6 have the highest correlation among cigarette brands at 0.998, suggesting nearly identical manufacturing or environmental PAH signatures. High significance across the board confirms the 12 PAHs are reliable markers for tobacco products. high levels of one "Class 1" carcinogen like Chrysene, implies elevated levels of other PAHs

Table 4.2: TS1-7 (Tobacco cigarette brands + TS8 Tobacco leaves) GC-MS Chemical Compounds

Chemical Class	TS1 (26)	TS2 (19)	TS3 (28)	TS4 (21)	TS5 (16)	TS6 (25)	TS7 (12)	TS8 (27)
Monoterpene Der.	7.26	6.69	2.20	8.25	6.03	2.62	14.54	6.90
TSNA	23.96	31.23	25.11	34.46	33.17	22.5	18.96	7.40
Diterpene	4.09	4.92	3.77	6.72	7.65	4.51	3.21	5.06

Fatty Acid - Amide	3.83	6.13	5.76	7.41	7.61	17.6	4.01	6.08
Aromatic Abietane Diterpene	3.15	2.35	2.01	2.05	2.05	2.07	*	2.08
Phenylpropanoid ester	*	*	1.22	*	*	1.86	*	*
Alkyl/Hydroxyl PAH	1.33	*	2.56	1.80	*	3.63	*	1.34
Ester	1.57	17.20	6.96	6.01	6.54	17.18	*	9.49
Phathalate	*	1.93	4.13	1.68	*	1.36	*	*
Tobacco wax	41.07	9.29	20.13	20.60	26.76	16.12	10.77	34.75
TSNA-TMS	6.30	17.00	13.76	5.57	2.91	4.38	4.01	*
Sterol	3.89	3.25	4.17	5.51	7.29	4.79	2.79	1.52
Alkaloid	1.86	*	8.23	*	*	*	*	1.91
Alcohol		*	*	*	*	1.38	*	*
Phytosterol	1.68	*	*	*	*	*	41.71	23.48
Total / Sample	100.00	99.99	100.00	100.06	100.01	100.00	100.00	100.00

** = Not detected. Values represent relative abundance (% Max Intensity).*

Statistics: Spearman Correlation Matrix (rs)

	TS1	TS2	TS3	TS4	TS5	TS6	TS7	TS8
TS1	1.000	0.748	0.607	0.863	0.824	0.616	0.906	0.652
TS2	0.748	1.000	0.671	0.875	0.811	0.791	0.737	0.676
TS3	0.607	0.671	1.000	0.554	0.587	0.565	0.522	0.487
TS4	0.863	0.875	0.554	1.000	0.914	0.828	0.878	0.806
TS5	0.824	0.811	0.587	0.914	1.000	0.879	0.796	0.771
TS6	0.616	0.791	0.565	0.828	0.879	1.000	0.639	0.676
TS7	0.906	0.737	0.522	0.878	0.796	0.639	1.000	0.541
TS8	0.652	0.676	0.487	0.806	0.771	0.676	0.541	1.000

Spearman Correlation matrix p-values

	TS1	TS2	TS3	TS4	TS5	TS6	TS7	TS8
TS1	0.0000	0.0021	0.0213	0.0001	0.0006	0.0168	0.0000	0.0115
TS2	0.0021	0.0000	0.0086	0.0000	0.0006	0.0005	0.0027	0.0079

TS3	0.0213	0.0086	0.0000	0.0398	0.0495	0.0195	0.0557	0.0776
TS4	0.0001	0.0000	0.0398	0.0000	0.0000	0.0002	0.0000	0.0005
TS5	0.0006	0.0006	0.0495	0.0000	0.0000	0.0001	0.0008	0.0022
TS6	0.0168	0.0005	0.0195	0.0002	0.0001	0.0000	0.0137	0.0067
TS7	0.0000	0.0027	0.0557	0.0000	0.0008	0.0137	0.0000	0.0459
TS8	0.0115	0.0079	0.0776	0.0005	0.0022	0.0067	0.0459	0.0000

Toxin and PAH Load Comparison

The manufactured cigarette brands (TS1–TS6) generally carry a much higher toxicant burden than the raw tobacco leaf (TS8).

Sample	Toxin Load (%)*	PAH Load (%)	Primary Toxin Source
TS1	30.78%	1.35%	Tobacco wax (41.78%)
TS2	50.17%	0.00%	TSNA + TSNA-TMS (48.23%)
TS3	43.00%	2.56%	TSNA + TSNA-TMS (38.87%)
TS4	41.68%	1.80%	TSNA (34.44%)
TS5	36.08%	0.00%	TSNA (33.17%)
TS6	28.24%	3.63%	TSNA (22.50%)
TS7	39.41%	0.00%	TSNA (32.53%)
TS8	9.67%	1.75%	Tobacco wax (45.41%)

**Toxin Load includes TSNA, TSNA-TMS, and Phthalates.*

De Novo Structural Deduction of TS1-TS8

The chemical composition of tobacco products TS1-TS8 reveals varying levels of compounds across samples.

TSNA (tobacco-specific nitrosamines) ranges from 7.40% (TS8) to 34.46% (TS4), with NNK-TMS derivatives also present (2.91-17.00%). Tobacco wax content varies significantly (9.29-41.07%), while phytosterol is prominent in TS7 (41.71%). Monoterpene derivatives, diterpenes, and fatty acid amides are also detected across samples. Carcinogenic PAHs are present in some samples (1.33-3.63%). While TSNA-NNK dominated TS1 to TS8. Alkaloids and sterols show sample-specific distributions. The table ideally confirms that nicotine a natural alkaloid in tobacco is the major percussor for NNK formation during tobacco curing.

Butadiene-menthol are industrial markers for Nigerian tobacco products, with M+ of 207. The dominance of 207 m/z is due to several factors, including prenyl monoterpene oxygenate and contributions from NNK. Like NNK, menthol or menthyl cation is highly reactive after losing a hydrogen atom and forms adducts with TMS or adds more isoprene units to its fragment, such as butadiene, as seen in TS1-TS7 where menthol forms varying adducts. The m/z 154 diagnostic ion, coupled with a high-confidence library hit, accurately identified the compound's structural core as menthol. Due to the non-polarity of this stationary phase, polar peaks, especially menthol

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derivatives, were the first eluates in TS1 to TS7. A base peak at m/z 81 confirms the cyclohexenyl ion.

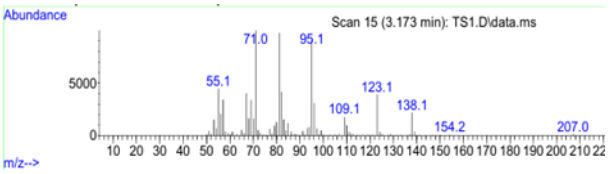

Figure 1: Butadiene-menthol

In TS1 cigarette brand, 41.07% tobacco wax, 23.96% TSNA, in addition to 6.30% NNK-TMS, and 7.26% mentholated flavouring agent were observed. In TS2 cigarette brand, 31.23% TSNA, in addition to 7.0% NNK-TMS, 17.20% fatty acid, 9.29% tobacco wax, and 6.69% mentholated flavouring agent were observed. In TS3 cigarette brand, 25.11% TSNA, in addition to 13.76% NNK-TMS, 20.13% tobacco wax, 5.76% fatty acid, and 8.25% mentholated flavouring agent were observed.

4-(Methyl-nitrosamine)-1-(3-pyridyl)-1-butanone represents the molecular ion at m/z 207, and the base peak at m/z 84 represents the N-methyl pyrrolidine (C5H11N). The number one carcinogenic tobacco-specific nitrosamine, NNK, was identified in all samples. The compound's structure was validated by its specific leaving group fragmentation ladder. A library hit identified the nicotine core due to m/z 161. However, a leaving group (NO from the nitrosamine group and O from the butanone bridge) perfectly matches the neutral leaving group, resulting in the open nicotine ring. The structural validation and fragmentation pattern match exactly that of the NNK compound. The fragmentation pattern further reveals that nicotine alkaloid is the precursor for most carcinogen derivatives in tobacco, apart from PAHs. During tobacco curing, Enterobacillus and Staphylococcus bacteria convert nitrate into nitrite, which reacts rapidly with nicotine in the leaves. The NNK biosynthetic pathway shows that nicotine is oxidized by breaking its pyrrole ring to form a pseudooxy-nicotine. Oxygenated nicotine is easily nitrosaminated by nitrite generated by bacteria into NNK. Nicotine is the precursor moiety to TSNA compounds. NNK is classified as a class 1 carcinogen. Both nicotine and NNK are highly toxic to humans. NNK is also very reactive and forms adducts with many other compounds.

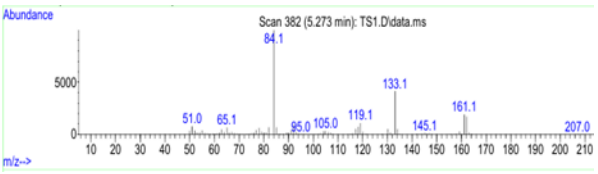

Figure 2: 4-(Methyl-nitrosamine)-1-(3-pyridyl)-1-butanone

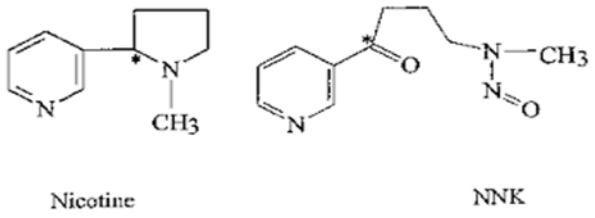

Figure 3a and 3b: Nicotine and NNK

A sequential loss of 14 (CH2) pattern from m/z 109 to m/z 222 was observed, indicating a long hydrocarbon chain. A C20 diterpene with 4 isoprene units was confirmed by its base peak at m/z 68 for Neophytadiene. This compound was found in all TS1-TS8, representing a perfect biomarker for tobacco products.

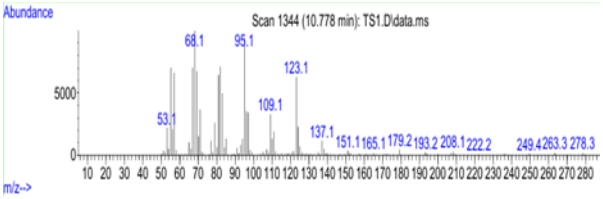

Figure 4: Neophytadiene mass spectrum

The stable molecular ion at m/z 281 and the core ion at m/z 243 suggest a resilient amide framework, while the base peaks at m/z 68 and 95 result from allylic cleavages facilitated by the double bond positions. The presence of m/z 60 and 73 diagnostic ions confirms the amide head group, while the lower-mass alkenyl ions (m/z 55, 97) reflect the eventual breakdown of the unsaturated hydrophobic tail.

Due to the high reactivity of carcinogens (NNK, PAHs, and nornicotine) at higher column temperatures, a complex compound of these toxins was observed. Late-eluting peaks showed a remarkable lipid-carcinogen trap co-eluting with varying tobacco waxes from C27 to C39. An in-situ reaction of NNK (207), hydroxy-nor-nicotine (165), and N-methyl-pyrrole-phenone TMS complexes

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characterized the late retention time as more non-polar compounds eluted.

The true molecular ion rule ensured exact GC-MS identification. Silyating reagent products reacting at higher temperatures were TMS-74, bis-TMS-148, bis-TMS-(benzene)-222, tris-TMS-(benzene)-296, and hydroxylated tris-TMS-(benzene)-314. Deduction of these masses from the observed M+ revealed the true mass for each peak. The carcinogens NNK (207), PAHs, alkaloids, and phenones with co-eluting waxes were accurately deduced and validated.

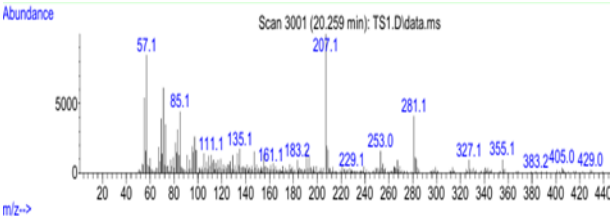

Figure 5: NNK-1,2-Bis(trimethylsilyl)benzene MS.

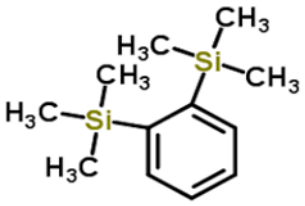

Figure 6: 1,2-Bis(trimethylsilyl)benzene

Table 4.2 Spearman correlation matrix:
Spearman r correlation was calculated using the formula: $rs = 1 - \frac{6\ Ed_i^2}{n(n^2-1)}$

Where di represent the difference between the ranks of chemical class for two different samples. While n is the number of chemical classes being compared, and p-value was calculated from t-distribution table with n – 2 degrees of freedom. TS4-TS5, TS4-TS2, and TS1-TS7 show extremely low p-values < 0.01. TS3 fails to show a significant correlation with TS7 ($p > 0.0557$) and TS8 ($p > 0.0776$) due to high percentage of alkaloids and Phenylpropanoid esters in this brand. TS4 and TS5 indicated $r_s = 0.914$ and $p < 0.00$, showing the strongest correlation. All tobacco brands TS1–TS6 are statistically significant (with $p < 0.05$), indicating a consistent chemical "fingerprint" across these products.

V. CONCLUSION AND POLICY RECOMMENDATIONS

5.1 Summary of Finding:

The concentration ranges of chemical classes in tobacco products TS1-TS8 reveal significant variability across samples. TS1 shows the widest spread (1.35-41.78%), dominated by tobacco wax, while TS8 has the highest individual peak (45.41%) also in tobacco wax. TS2 is notable for high TSNA concentrations (up to 31.23%), whereas TS6 has elevated levels of Fatty Acid Amides and Esters. TS3 stands out for having the most diverse chemical profile, albeit with the lowest maximum peak (25.11%). Other samples (TS4, TS5, TS7) show moderate concentration ranges. These variations highlight differences in composition among the tobacco products

- Highest Toxin Load: TS2 contains the highest percentage of toxins at 50.17%, largely due to a combined TSNA and TSNA-TMS profile.
- Highest PAH Load: TS6 stands out with 3.63% PAHs, nearly double the amount found in the raw tobacco leaf (1.75%).
- Natural vs. Processed: The raw leaf (TS8) has a toxin load of only 9.67%, roughly 3 to 5 times lower than the manufactured brands, though its PAH levels are comparable to many of the processed samples.

5.2 Policy Recommendations

1. Advanced Regulatory Monitoring: Nigerian regulatory bodies, specifically the National Agency for Food and Drug Administration and Control (NAFDAC) and the Standards Organisation of Nigeria (SON), should move beyond traditional "Nicotine/Tar" labeling. We recommend mandatory monitoring of TSNAs (NNK/NNN) and Priority PAHs as primary toxic markers for product registration.
2. Industrial Manufacturing Standards: Stricter "Good Manufacturing Practice" (GMP) guidelines must be enforced to eliminate the phthalate-wax adducts found in processed brands. These contaminants, likely stemming

from plasticizers in industrial machinery or sub-standard packaging, add an unnecessary layer of endocrine-disrupting toxicity.

3. Evidence-Based Public Health Campaigns: The Federal Ministry of Health should utilize the Stimulant-Toxin Ratio data from this study to inform public awareness. Campaigns should emphasize that the brief "relaxation" induced by nicotine comes at the cost of a massive, synergistic toxic load that is physically "trapped" and delivered by the cigarette's own wax matrix.

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