Toxicology of Ngirimbo Samples across Chitipa District

Vita Mithi (vitavmithi@gmail.com)
Armref Data for Action in Public Health Research

John F. Kamanula
Mzuzu University

Ashok Pandey
Policy Research Institute

Rebecca S. Dewey
University of Nottingham

Research

Keywords: Ngirimbo, Nicotine, Smokeless Tobacco, Toxicity, Heavy metals, Minerals

DOI: https://doi.org/10.21203/rs.3.rs-521155/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Toxicology of *Ngirimbo* Samples across Chitipa District

Vita Mithi, BPH\(^1\), John F. Kamanula, PHD\(^2\), Ashok Pandey MPH/BPH/DGH\(^3\) and Rebecca S. Dewey, PHD\(^4\)

\(^1\)Armref Data for Action in Public Health Research, Mbelwa road, Recreation Center, Mzuzu, Malawi; [dataphr@yahoo.com](mailto:dataphr@yahoo.com)

\(^2\)Department of Chemistry, Faculty of Science, Technology and Innovation, Mzuzu University, P/Bag 201, Luwinga, Mzuzu 2, Malawi.

\(^3\)Policy Research Institute (PRI), Sanogaucharan, Kathmandu, Nepal.

\(^4\)Sir Peter Mansfield Imaging Centre, School of Physics and Astronomy, University of Nottingham, United Kingdom.

Corresponding author: Vita Mithi; email: [vitavmithi@gmail.com](mailto:vitavmithi@gmail.com)

30\(^{th}\) April, 2021
Abstract

**Introduction:** Smokeless tobacco describes a wide variety of tobacco products that do not require combustion, and is typically used either orally or nasally. *Ngirimbo* is a form of oral smokeless tobacco used by smokers in Malawi for tobacco harm reduction. The aim of this study was to determine the acidity (pH), nicotine content, mineral content (iron, zinc, calcium, magnesium and copper), heavy metal content (lead, chromium and cadmium), and presence of other volatile compounds in *ngirimbo* across Chitipa District in Malawi.

**Methods:** Atomic absorption spectrophotometry and gas chromatography-mass spectrometry were used to estimate nicotine content, concentration of toxic heavy metals, minerals of potential toxicity and other harmful chemicals in the samples.

**Results:** Samples were found to contain harmful chemicals, high pH and nicotine [2-(1-methyl-2-pyrolidinyl)-pyridine, (S)- and (S)-3-(1-methyl-2-pyrolidinyl)-pyridine] levels. Mineral concentrations were found to be much higher than typical safety limits. Conversely, samples were not found to contain lead, and had low concentrations of chromium and cadmium.

**Conclusions:** These findings suggest that prolonged use of *ngirimbo* is a significant health risk to people with chronic diseases. Nonetheless, *ngirimbo* provides a valid method of tobacco harm reduction and a potential smoking cessation tool. Therefore, further analytical toxicological studies are needed to fully characterize variations in the quality of the product.

**Implications:** Consumers of *ngirimbo* are susceptible to acute toxic effects of oral and dermal exposure to the product, as well as addiction. Some individuals would suffer from convulsions or seizures following *ngirimbo* use, and other clinical consequences depending on the amount ingested. However, understanding the amount of nicotine, and other volatile constituents, consumed through *ngirimbo* use will help to generate recommendations for quantity and frequency of use. Further, establishing the threshold of harm for nicotine consumption will contribute to the extraction, isolation, and use of nicotine as a smoking-cessation agent, and for treating psychiatric disorders such as schizophrenia and neurodegenerative diseases such as Alzheimer’s.

**Keywords:** *Ngirimbo*, Nicotine, Smokeless Tobacco, Toxicity, Heavy metals, Minerals
Introduction
The term smokeless tobacco describes a wide variety of tobacco products which do not require combustion. These products are typically used orally or nasally. When used orally, tobacco is predominantly sucked (as dry or moist snuff), or chewed (chewing tobacco), whereas tobacco for nasal use is sniffed (dry snuff). *Ngirimbo* is a form of smokeless tobacco used orally in Malawi. The product is considered to be a method of tobacco harm reduction as it is safer in comparison to cigarettes due to the absence of combustion/burning during consumption. *Ngirimbo* is made from a mixture of *ilambo* (a crystalline solid comprised of plant remains such as stalk or husks), cold water, and local tobacco.

According to the Malawi National STEPwise Survey for Non-Communicable Diseases Risk Factors 2017 Report, tobacco use is increasingly becoming a significant cause of mental health problems, morbidity, and mortality among adults and young people in Malawi. There is no current infrastructure in Malawi providing assistance for tobacco cessation, nor a widespread availability of safer nicotine products or nicotine replacement therapies such as patches or gum at affordable prices. The general population favors cheaper local smokeless tobacco products to reduce the harm caused by smoking and nicotine addiction. Reportedly, 0.4% of Malawians use smokeless tobacco products \(^1\). Despite this relatively low popularity of smokeless tobacco use, it represents an ongoing public health challenge that is amplifying the levels of drug and substance exploitation and mental illness in Malawi. Furthermore, very little is known about the potential toxicity or use of locally-made tobacco harm reduction products. This study aims to provide an understanding of *ngirimbo* use, characterizing the properties of the product, while assessing the prospect of utilizing *ngirimbo* in more wide-reaching tobacco harm reduction programs.

Materials and Methods
Samples of *ngirimbo* were collected from producers in twelve different areas of Chitipa district. Sources were selected based on the popularity of the producers in terms of the number of local markets per area, thereby representing a large and uniform sample group. Samples were personally collected from the producers in their original vacuum-
packed containers, and labeled with unique identification codes to signify the ngirimbo production area (GPA), and labeled GPA1-12 for ease of reference.

**Determination of sample pH**
The pH of each powdered sample of ngirimbo was measured using a pH meter (Denver Instrument, Basic pH meter, 10487; 12V, 500mA, USA) that had been previously calibrated using two buffer solutions (at pH 4.00 and 7.00) prior to testing. A 10.0 g sample of each powder was weighed using an analytical balance (AE ADAM, PW 214, max wt. 210 g) in triplicate. Distilled water (100 mL) was added to the sample and stirred using a glass rod for about 5 minutes prior to being introduced to the pH meter, and the pH value of the sample being read from the screen. Statistical analyses were conducted using Statistical Package for the Social Sciences (SPSS) to compare the mean pH values across samples.

**Assessment of mineral and heavy metal content**
Ngirimbo samples underwent analysis using atomic absorption spectrophotometry (AAS) for the presence of iron (Fe), zinc (Zn), calcium (Ca), magnesium (Mg), copper (Cu), and heavy metals, lead (Pb), chromium (Cr), and cadmium (Cd). Each sample was treated with 6 mL of 6 M hydrochloric acid, HCl (aq) and dried on a hot plate. After cooling, 10 mL of 6 M HCl (aq) was added to the residue in the crucible and heated to boiling using a hot plate. The sample was cooled at ambient temperature and filtered quantitatively into a flask. The solution was diluted to the 100 mL mark with distilled water. A control sample was also treated in the same way as the samples, but contained only the reagents added to the sample and no ngirimbo.

**Calculation of calibration curves**
In order to calculate the calibration curves for each element of interest, standard samples were prepared for Fe, Zn, Ca, Mg, Cu, Pb, Cr, and Cd. This was conducted using a 1000 mg/L stock solution of each metal solution, whereby standards were prepared at serial dilutions of 0 (control), 0.1, 0.2, 0.5, 1.0, 1.5, 2.0, 2.5 and 5.0 mg/L for each element.
**Analysis of ngirimbo samples**

Each standard and sample (including the control sample) underwent AAS following the manufacturer’s protocol. The results are presented in mg/100g sample which was obtained using the following formula:

\[
\text{Concentration in mg/100 g of a sample} = \frac{[(a-b) \times V]}{10 \times W}
\]

Where:
- \(a\) = Concentration of sample (mg/L)
- \(b\) = Concentration of control (mg/L)
- \(V\) = Volume used (100 mL)
- \(W\) = Mass of fresh sample (g)

**Assessment of volatile compound content**

Samples underwent gas chromatography-mass spectrometry (GC-MS) analysis to determine the presence and concentration of volatile compounds. 1.5 g of each powdered sample was accurately weighed using an analytical balance (AE ADAM, PW 214, max wt. 210 g). Distilled water (20 mL) was added to each sample, followed by 40 mL of n-Hexane, and finally 10 mL of 2 M sodium hydroxide, NaOH (aq). The mixture was stirred for about 5 minutes using a glass rod. The sample was transferred into a separating funnel and shaken thoroughly for about 5 minutes, before being left to stand for about 20 minutes until two distinct layers formed. The aqueous (bottom) layer was removed and the organic n-hexane (top) layer was transferred into a stoppered conical flask. Anhydrous sodium sulphate, Na\(_2\)SO\(_4\) (s) was added as an inert drying agent. The sample was filtered into a 2 mL vial using a 0.45µm filter membrane (nylon syringe filter).
Results

Sample pH
The pH level of the 12 samples was found to range from 7.5 to 10.7 with a mean of 9.2. Sample GPA6 had the highest pH of 10.7 and sample GPA7 gave the lowest value of 7.5.

Table 1a: ANOVA of sample pH

|                  | Sum of Squares | df | Mean Square | F      | Sig. |
|------------------|----------------|----|-------------|--------|------|
| Between Groups   | 28.005         | 11 | 2.546       | 825.696| .000 |
| Within Groups    | .074           | 24 | .003        |        |      |
| Total            | 28.079         | 35 |             |        |      |
Table 1b: *Ngirimbo* pH; post-hoc tests

Post Hoc Tests

**Homogeneous Subsets**

| Sampling point | N  | pH | Subset for alpha = 0.05 |
|----------------|----|----|------------------------|
|                |    | 1  | 2          | 3  | 4  | 5  | 6  | 7  | 8  |
| GPA 1          | 3  | 9.7500      |            |    |    |    |    |    |    |
| GPA 2          | 3  | 9.7567      |            |    |    |    |    |    |    |
| GPA 3          | 3  | 9.7833      | 9.7833     |    |    |    |    |    |    |
| GPA 4          | 3  | 9.0133      |            |    |    |    |    |    |    |
| GPA 5          | 3  | 9.4267      |            |    |    |    |    |    |    |
| GPA 6          | 3  | 10.6867     |            |    |    |    |    |    |    |
| GPA 7          | 3  | 7.5033      | 7.6800     |    |    |    |    |    |    |
| GPA 8          | 3  | 7.6800      |            |    |    |    |    |    |    |
| GPA 9          | 3  | 8.6700      |            |    |    |    |    |    |    |
| GPA 10         | 3  | 8.9067      |            |    |    |    |    |    |    |
| GPA 11         | 3  |            |            |    |    |    |    |    |    |
| GPA 12         | 3  |            |            |    |    |    |    |    |    |

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

**KEY:** GPA = Ngirimbo Production Area
Concentration of metallic ions in *ngirimbo*.
The concentrations of metallic ions, Fe, Zn, Ca, Mg, and Cu, within *ngirimbo* samples are expressed in Table 2, in units of mg/100g.

**Table 2: Concentrations of heavy metals in *ngirimbo***

| Sample  | Fe (mg/100 g sample) | Zn (mg/100 g sample) | Ca (mg/100 g sample) | Mg (mg/100 g sample) | Cu (mg/100 g sample) |
|---------|----------------------|----------------------|----------------------|----------------------|----------------------|
| GPA 1   | 186.73               | 3.68                 | 1065.27              | 178.08               | 0.94                 |
| GPA 2   | 301.84               | 4.10                 | 752.26               | 321.16               | 2.11                 |
| GPA 3   | 409.02               | 5.26                 | 970.95               | 460.36               | 2.43                 |
| GPA 4   | 402.77               | 5.35                 | 1213.12              | 553.36               | 3.77                 |
| GPA 5   | 379.07               | 4.85                 | 794.04               | 396.00               | 2.06                 |
| GPA 6   | 223.12               | 4.15                 | 715.17               | 428.00               | 2.22                 |
| GPA 7   | 357.32               | 5.61                 | 1047.74              | 418.44               | 2.68                 |
| GPA 8   | 300.04               | 5.10                 | 1893.84              | 744.04               | 3.05                 |
| GPA 9   | 159.21               | 3.21                 | 801.13               | 264.26               | 1.89                 |
| GPA 10  | 232.48               | 2.80                 | 462.51               | 233.92               | 1.58                 |
| GPA 11  | 276.18               | 3.07                 | 672.17               | 201.52               | 1.43                 |
| GPA 12  | 196.44               | 1.74                 | 265.18               | 113.96               | 0.97                 |

**KEY**
- mg = Milligram
- g = Gram
- GPA = Ngirimbo Production Area
Concentrations of heavy metals
Various heavy metals are categorized by the International Agency for Research on Cancer (IARC) as group 1 and 2 carcinogenic metals, signifying that they are known and probable human carcinogens. Cadmium and chromium are classified as group 1, and lead as group 2. The presence of these metals in *ngirimbo* was of particular interest in this study as it poses a potential threat to humans. Cadmium and chromium levels varied from 0.00mg/100g to 0.89gm/100g in all samples while lead was not detected in any of the samples.

**Table 3: Concentrations of heavy metals**

| Sample  | Pb (mg/100 g sample) | Cr (mg/100 g sample) | Cd (mg/100 g sample) |
|---------|----------------------|----------------------|----------------------|
| GPA 1   | Nd                   | 0.10                 | 0.13                 |
| GPA 2   | Nd                   | 0.57                 | 0.03                 |
| GPA 3   | Nd                   | 0.76                 | 0.04                 |
| GPA 4   | Nd                   | 0.76                 | 0.06                 |
| GPA 5   | Nd                   | 0.62                 | 0.05                 |
| GPA 6   | Nd                   | 0.56                 | 0.03                 |
| GPA 7   | Nd                   | 0.81                 | 0.04                 |
| GPA 8   | Nd                   | 0.31                 | 0.07                 |
| GPA 9   | Nd                   | 0.33                 | 0.05                 |
| GPA 10  | Nd                   | 0.89                 | 0.02                 |
| GPA 11  | Nd                   | 0.89                 | 0.02                 |
| GPA 12  | Nd                   | 0.58                 | 0.00                 |

**KEY**  
Nd = Not detected  
mg = Milligram  
g = Gram  
GPA = Ngirimbo Production Area
Nicotine concentration
Nicotine concentration varied from 0.97% to 2.79% across all samples, above the standard sample (for comparison, nicotine gum typically reports a concentration of 0.32%).

Table 4: Nicotine concentration in *ngirimbo*

| Sample | RT (min) | Nicotine in Ngirimbo (% PA) | Nicotine STD (%PA) | Nicotine STD (mg) | Ngirimbo (mg) | Nicotine in Ngirimbo (g) | Mass of sample (g) | Nicotine in sample (%) |
|--------|---------|----------------------------|--------------------|-------------------|---------------|-------------------------|-------------------|----------------------|
| GPA 1  | 10.167  | 20.12                      | 3.19               | 4                 | 25.2288       | 0.02523                 | 1.5004            | 1.68                 |
| GPA 2  | 10.146  | 20.28                      | 3.19               | 4                 | 25.4295       | 0.02543                 | 1.5034            | 1.69                 |
| GPA 3  | 10.125  | 11.68                      | 3.19               | 4                 | 14.6458       | 0.01465                 | 1.5020            | 0.98                 |
| GPA 4  | 10.167  | 31.24                      | 3.19               | 4                 | 39.1724       | 0.03917                 | 1.5003            | 2.61                 |
| GPA 5  | 10.146  | 21.64                      | 3.19               | 4                 | 27.1348       | 0.02713                 | 1.5030            | 1.81                 |
| GPA 6  | 10.146  | 21.82                      | 3.19               | 4                 | 27.3605       | 0.02736                 | 1.5010            | 1.82                 |
| GPA 7  | 10.167  | 27.29                      | 3.19               | 4                 | 34.2194       | 0.03422                 | 1.5007            | 2.28                 |
| GPA 8  | 10.167  | 33.34                      | 3.19               | 4                 | 41.8056       | 0.04181                 | 1.5002            | 2.79                 |
| GPA 9  | 10.146  | 23.02                      | 3.19               | 4                 | 28.8652       | 0.02887                 | 1.5003            | 1.92                 |
| GPA 10 | 10.167  | 20.7                       | 3.19               | 4                 | 25.9561       | 0.02596                 | 1.5003            | 1.73                 |
| GPA 11 | 10.146  | 20.29                      | 3.19               | 4                 | 25.4420       | 0.02544                 | 1.5007            | 1.70                 |
| GPA 12 | 10.125  | 11.65                      | 3.19               | 4                 | 14.6082       | 0.01461                 | 1.5020            | 0.97                 |
| Nicotine STD | 10.187 | 3.19                      | 3.19               | 4                 | 4.0000        | 0.00400                 | 1.2452            | 0.32                 |

**KEY**
- GPA = Ngirimbo Production Area
- PA = Peak Area
- g=Gram
- %= Percentage
- STD=Standard
- RT = Retention time
Volatile compounds in *ngirimbo*

*Ngitrimbo* powder is a mixture of substances, containing a variety of organic volatile compounds as presented in Tables 1 to 12 in supplementary material. *Ngirimbo* was found to contain high levels of 2-(1-methyl-2-pyrolidinyl)-pyridine, (S) - and (S)-3-(1-methyl-2-pyrolidinyl)-pyridine (Nicotine) with peak area percentages (PA %) ranging from 11.65% to 33.34% across all samples in the study.

**Discussion**

Previous findings suggest that the total nicotine content in local smokeless tobacco products (LSTP) such as *ngirimbo* is the primary determinant of product consumption and consumer attractiveness. The product pH is a significant element to the net nicotine dose obtainable from using these products. A variety of volatile compounds (Tables 1 – 12) found in the *ngirimbo* samples analyzed in this study show a variation in product acidity, which may in turn modulate levels of nicotine absorption and toxicity. This may create an avenue for manufacturer competition for consumer preference, and also impact the potential for addiction. Studies indicate that smokeless tobacco products contain greater levels of nicotine and carcinogenic compounds in comparison to nicotine gum. These findings are in agreement, in it can be concluded that *ngirimbo* contains harmful chemicals, high pH and nicotine levels as shown in Table 1b, and Table 4, and Tables 1-12 of Supplementary materials.

Despite the fact that nicotine is widely used recreationally as a stimulant and anxiolytic, and is highly addictive to the central nervous system (CNS), it is relevant to note the types of products in use, their method of administration, and the quantity of nicotine being consumed. These are important considerations in the context of potential toxicity to an individual. *Ngirimbo* contains nicotine, which itself is categorized by the International Agency for Research on Cancer (IARC) as an environmental hazard, with the ability to cause acute, oral and dermal toxic effects. Animal studies have shown that the compound has the ability to induce addiction, increase cardiac blood pressure, and effects such as convulsions or seizures. Nicotine is mainly found in alkaloid plants, and is typically manufactured for human use in the form of the leaves of the tobacco plant.

2-(1-methyl-2-pyrolidinyl)-pyridine, (S)- is a compound that contains alpha-nicotine (α-nicotine). Alpha-nicotine is a nicotine analog that has a high affinity for nicotine receptors. It has the ability to mediate the release of neurotransmitters, which makes it a good candidate for treating psychiatric disorders, such as schizophrenia, and neurodegenerative diseases, such as Alzheimer’s disease. In addition, it can be used as a smoking cessation agent. Animal studies have shown that the compound has no toxic effects. Therefore, the use of alpha-nicotine could be used to replace the
use of *ngirimbo* and other conventional nicotine products to reduce the toxicity of substances consumed, depending on type of nicotine product taken and the level of intake.

Furthermore, these analysis found that *ngirimbo* samples contained high levels of trace metals of clinical importance (see Table 2), as well as low levels of heavy metals (Table 3). Assessing the concentrations of these metals present in *ngirimbo* is highly relevant for assessing the safety of the product as these metals are involved in regulating normal physiological processes in the human body. Increasing the concentrations of these metals beyond the physiologically normal range has the capacity to cause harm to human health.\(^\text{13}\).

Iron, copper, and zinc are essential minerals. The recommended daily intake of iron is 8-18g, copper 0.9mg, and zinc 8-11g.\(^\text{14}\) Excessive iron intake may lead to build up of iron in tissues and organs, exacerbating hemochromatosis,\(^\text{15}\) while high copper intake can cause functional damage to organs such as the liver.\(^\text{16}\) Zinc can suppress the function of the immune system,\(^\text{17}\) but conversely can aid in the treatment of depression.\(^\text{17}\)

Magnesium plays useful roles in reducing hypertension, and improving insulin sensitivity and lipid profiles in patients at risk of cardiovascular diseases. Nonetheless, a high magnesium level of 2.6 mg/dL or above for a prolonged time can cause hypermagnesemia and associated toxic effects.\(^\text{18}\) Calcium is important in bone development, however, too much calcium can cause hypercalcemia.\(^\text{19}\)

In this study, the samples tested showed concentrations exceeding the recommended daily intake levels of copper, magnesium, zinc, calcium, and iron. This is likely to be due to environmental, occupational, biological, and geographic sources of these elements resulting in build-up within the tobacco plants. It is important to note that the range of typical daily *ngirimbo* intake for consumers has not been established. Further, toxicity levels for *ngirimbo* and many of its contaminants have not yet been determined.

Tobacco has been identified as a source for many heavy metals such as cadmium, chromium, and lead.\(^\text{20}\) Animal studies have shown cadmium to be a cytotoxic agent with the potential to cause cancer and hemolysis even in low availability.\(^\text{21}\) Through animal, toxicological, human, and epidemiological studies, it has been shown that hexavalent chromium is toxic and carcinogenic, with any amount of hexavalent chromium entering cells having the potential to initiate tumor formation.\(^\text{22}\) Conversely, trivalent chromium has been proposed as a dietary supplement, with the ability to induce insulin sensitivity in humans with insulin resistance (pre-diabetes), and improve impaired blood glucose tolerance.\(^\text{23,24}\) It is necessary to note that *ngirimbo* consumption may increase the risk of hexavalent chromium or trivalent chromium intake and toxicity when other contributing sources of chromium are considered. Aside from the heavy metals mentioned above, the present study has
detected no presence of lead in *ngirimbo* (Table 3). This provides some reassurance that LSTP use is unlikely to cause complications associated with lead consumption.

In addition, it should be noted that the extent and degree of metal accumulation in plant products is determined by the geographical region of cultivation, climatic circumstances, soil pH and chemistry. As such, human exposure to higher volumes of the metallic ions detected in *ngirimbo* may cause different clinical side effects depending on the baseline levels of the compound, the quantity absorbed or consumed through *ngirimbo* use, and on the target organ.

**Conclusion**

The results of this analysis demonstrate that harmful volatile chemical compounds in *ngirimbo* are catalysts that elevate acidity levels, toxicity, and the carcinogenic capacities of cadmium and nicotine associated with *ngirimbo* use. Conversely, the analysis suggests that the nicotine antagonist and a potential smoking cessation agent, 2-(1-methyl-2-pyrolidinyl)-pyridine, (S), is available in *ngirimbo*. These findings demonstrate the need for an in-depth analysis of the raw materials that are used in the production of *ngirimbo*. This information will then be critical to inform the widespread use of *ngirimbo* as a smoking cessation aid. Trace metals and chromium must be minimized to avoid toxicity and risk to human health when dietary and environmental exposures are also taken into consideration.

In view of this evidence, it is recommended that the production of LSTP should be placed under quality control procedures according to national guidelines in Malawi, and that producers be given licenses under government observation. Furthermore, it is necessary to refine production methods to reduce the incidence of potential addictive and toxic compounds in *ngirimbo* such that it can be recognised as a tobacco harm reduction product. Consequently, future research is needed to determine recommended dosing and precautionary measures, with concrete evidence of the adverse effects on living organisms, such that this information can be published on the label of the product.

Consequently, the findings of this study provide an impetus for further research, as follows:

- To determine population behaviour regarding the quantities of *ngirimbo* consumed, and the concentrations of nicotine obtained from varying levels of use.
- Potential toxicity with varying levels of *ngirimbo* use.
- Whether there is any relationship between *ngirimbo* consumption and the absorption of metal ions.
- A reconnaissance survey of *ngirimbo* use.
Abbreviations
AAS    Atomic absorption spectrophotometer
CNS   Central nervous system
GC-MS  Gas chromatography-mass spectrometry
GPA    Ngirimbo production area
IARC   International Agency for Research on Cancer
LSTP   Local smokeless tobacco products
pH     Potential of hydrogen
SPSS   Statistical Package for the Social Sciences

Funding
This study was funded by Knowledge Action Change. The funder did not take any part in designing
the study, methods, collection, analysis and interpretation of data or in writing the manuscript.

Declaration of Interests
There are no competing interests.

Availability of data and materials
All raw and analyzed data are available from the author upon request.

Authors’ contributions
VM composed the proposal, implemented the research, conducted data collection and analysis while
JK conducted sample analysis. RD worked on language, drafts and content of the paper. AP worked
on the discussion and reference. The final manuscript was read and approved by authors.

Corresponding author
Correspondence to Vita Mithi.

Competing interests
The authors have no competing interests.
Consent for publication
Not applicable.

Ethics approval and consent to participate
Not applicable

Acknowledgments
I am thankful to God for his favour and grace of knowledge and wisdom to accomplish this research. I convey my gratitude to Knowledge Action Change (KAC), for funding this research through the Tobacco Harm Reduction Scholarship Programme. Special thanks to Associate Professor Dr. John F. Kamanula head of chemistry at Mzuzu University for extending his valuable technical support for sample extraction and calculations, Idris Mtewa University of Malawi, Chancellor college for the GC/MS and AAS analysis, Charles Nkukumila of University of Malawi, Chancellor college and Steven Kaunda of Mzuzu University for sample preparations and organization of laboratory materials. Donald Nyondo, and Precious Mithi University of Livingstonia for data collection, Alfred Munkhondia natural leader for language interpretation with the indigenous community.

Special thanks to, Professor Terry Sunderland PhD., Faculty of Forestry University of British Columbia, Vancouver, Canada, Jon Derricott, Tobacco Harm Reduction Scholarship Manager, Knowledge Action Change, Dr. John Ramsey (retired analytical toxicologist) and Professor Rajesh N. Sharan, PHD., of North-Eastern Hill University, India, for the guidance and mentorship throughout the research.
References

1. Drope, Schluger, Cahn, Drope, Hamill, Islami, Liber, Nargis S. The Tobacco Atlas. Atlanta: American Cancer Society and Vital Strategies.; 2018. www.tobaccoatlas.org

2. World Health Organization & WHO Tobacco Free Initiative. Standard operating procedure for determination of nicotine in cigarette tobacco filler. Published online 2014:15. http://www.who.int/about/licensing/copyright_form/en/index.html

3. Prabhakar V, Jayakrishnan G, Nair S V, Ranganathan B. Determination of Trace Metals, Moisture, pH and Assessment of Potential Toxicity of Selected Smokeless Tobacco Products. Indian J Pharm Sci. 2013;75(3):262-269. doi:10.4103/0250-474X.117398

4. Lauterbach JH, Bao M, Joza PJ, Rickert WS. Free-base nicotine in tobacco products. Part II. Determination of free-base nicotine in the aqueous extracts of smokeless tobacco products and the relevance of these findings to product design parameters. Regul Toxicol Pharmacol. 2011;59(1):8-18. doi:10.1016/j.yrtph.2010.09.002

5. Ayo-Yusuf OA, Swart TJP, Pickworth WB. Nicotine delivery capabilities of smokeless tobacco products and implications for control of tobacco dependence in South Africa. Tob Control. 2004;13(2):186—189. doi:10.1136/tc.2003.006601

6. Smokeless tobacco and some tobacco-specific N-nitrosamines. IARC Monogr Eval Carcinog risks to humans. 2007;89:1-592.

7. Lindson N CSCYWFTRBC, Hartmann-Boyce J. Different doses, durations and modes of delivery of nicotine replacement therapy for smoking cessation. Cochrane Database Syst Rev. 2019;(4). doi:10.1002/14651858.CD013308

8. National C for Biotechnology information. PubChem Compound Summary for CID 157672, (+)-Nicotine. In: ; 2021. https://pubchem.ncbi.nlm.nih.gov/compound/3_-2R_-1-methylpyrrolidin-2-yl_pyridine. Accessed Mar. 23, 2021.

9. National Center for Biotechnology Information. PubChem Compound Summary for CID 89594, Nicotine . https://pubchem.ncbi.nlm.nih.gov/compound/Nicotine. Accessed Mar. 28, 2021.

10. National Center for Biotechnology information. Compound Summary for CID 942, 3-{1-Methylpyrrolidin-2-yl}pyridine. https://pubchem.ncbi.nlm.nih.gov/compound/3-_1-Methylpyrrolidin-2-yl_pyridine. Accessed Mar. 9, 2021.

11. National Center for Biotechnology information. PubChem Compound Summary for CID 212128, Pyridine, 2-(1-methyl-2-pyrrolidinyl)-. Published online 2021. https://pubchem.ncbi.nlm.nih.gov/compound/Pyridine_-2-_1-methyl-2-pyrrolidinyl. Accessed Mar. 9, 2021.

12. Rowland NE, Robertson K, Soti F, Kem WR. Nicotine analog inhibition of nicotine self-administration in rats. Psychopharmacology (Berl). 2008;199(4):605-613. doi:10.1007/s00213-008-1186-8

13. Bernhard D, Rossmann A, Wick G. Metals in cigarette smoke. IUBMB Life. 2006;57:805-809. doi:10.1080/15216540500459667

14. Trumbo P, Yates AA, Schlicker S, Poos M. Dietary reference intakes: vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. J Am Diet Assoc. 2001;101(3):294-301. doi:10.1016/S0002-8223(01)00078-5

15. Kowdley K V. Iron, hemochromatosis, and hepatocellular carcinoma. Gastroenterology.
16. Bisaglia M, Bubacco L. Copper Ions and Parkinson’s Disease: Why Is Homeostasis So Relevant? *Biomolecules*. 2020;10(2). doi:10.3390/biom10020195

17. Yary T, Aazami S. Dietary intake of zinc was inversely associated with depression. *Biol Trace Elem Res*. 2012;145(3):286-290. doi:10.1007/s12011-011-9202-y

18. Hadjistavri LS, Sarafidis PA, Georgianos PI, et al. Beneficial effects of oral magnesium supplementation on insulin sensitivity and serum lipid profile. *Med Sci Monit Int Med J Exp Clin Res*. 2010;16(6):CR307-312.

19. Ross AC, Taylor CL, Yaktine AL, Del Valle HB, eds. *Dietary Reference Intakes for Calcium and Vitamin D*; 2011. doi:10.17226/13050

20. Ashraf MW. Levels of Heavy Metals in Popular Cigarette Brands and Exposure to These Metals via Smoking. Canário J, Schnorr TM, eds. *Sci World J*. 2012;2012:729430. doi:10.1100/2012/729430

21. Mrugesh T, Dipa L, Manishika G. Effect of lead on human erythrocytes: an in vitro study. *Acta Pol Pharm*. 2011;68(5):653-656.

22. Sun H, Brocato J, Costa M. Oral Chromium Exposure and Toxicity. *Curr Environ Heal Reports*. 2015;2(3):295-303. doi:10.1007/s40572-015-0054-z

23. Witt KL, Stout MD, Herbert RA, et al. Mechanistic insights from the NTP studies of chromium. *Toxicol Pathol*. 2013;41(2):326-342. doi:10.1177/0192623312469856

24. Krejpcio Z. Essentiality of Chromium for Human Nutrition and Health. *Polish J Environ Stud*. 2001;10.

25. Rustemeier K, Stabbert R, Haussmann H-J, Roemer E, Carmines EL. Evaluation of the potential effects of ingredients added to cigarettes. Part 2: Chemical composition of mainstream smoke. *Food Chem Toxicol*. 2002;40(1):93-104. doi:https://doi.org/10.1016/S0278-6915(01)00085-0
Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- NgirimboResearchPaper.SupplementaryMaterial.pdf