

RESEARCH ARTICLE

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Comparative phytochemical analysis of aqueous leaf extracts of locally grown and imported variants of *Nicotiana Tabacum* in Southeastern Nigeria

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Abstract

Background The concentrations of phytochemical constituents in a plant are affected by the environmental factors, cultivation methods and diseases. The specific objective of the study was to analyze and compare the phytochemical composition of dried leaf extracts of foreign and Nigerian locally-grown varieties of *Nicotiana tabacum*.

Methods The study adopted an experimental research design consisting of laboratory assays of tobacco products marketed in Enugu metropolis, Southeast Nigeria. Two major sources of tobacco were identified during a preliminary survey of tobacco markets in Enugu metropolis. These were A, locally-grown tobacco obtained from Southwestern Nigeria and B, foreign-grown and processed tobacco leaves. Dry leaves of tobacco purchased from the two sources were analyzed for compositions, qualitative and quantitative phytochemical contents.

Results The local *Nicotiana tabacum* leaf extract had higher absolute and relative concentration of Anthracyanin (10.18 ± 1.79 mg/kg; versus 4.99 ± 0.75 mg/kg), Cyanogenic glycoside (7.88 ± 1.64 mg/kg; 4.86 ± 0.43 mg/kg), Rutin (11.92 ± 1.65 ; 4.98 ± 0.87 mg/kg), and Flavonones (7.01 ± 0.76 mg/kg; 3.55 ± 0.41 mg/kg). On the other hand, the foreign tobacco was found to have greater content of Cardiac glycoside, Steroids, Epicatechin, Phytate and Oxalate (7.93 ± 0.59 ; 4.26 ± 0.88 mg/kg, 10.99 ± 1.85 ; 6.11 ± 0.38 mg/kg, 21.00 ± 2.18 ; 9.82 ± 1.20 mg/kg, 8.98 ± 1.50 ; 4.40 ± 0.54 mg/kg, and 23.49 ± 2.58 ; 12.94 ± 1.31 mg/kg, respectively for foreign and local tobacco leaf extracts; $p=0.0001$). Some components were however, exclusively specie specific for the foreign tobacco (Lunamarin, Naringin, Flavon-3-ol, Naringenin) and the local tobacco (Proanthocyanin, Tanin, Flavone, Resveratrol), being virtually undetected in the other species. Oxalates are the most abundant in both foreign and local extracts (17.06%; 10.77%), followed in decreasing order by Epicatechin (15.30%; 8.19%) and Sapogenin (9.93%; 8.34%).

Conclusion The observed significantly high abundance of phytochemical constituents in the Nigerian locally-grown *Nicotiana tabacum* leaf extract in this study underscores the potentially rich medicinal, industrial and economic value of this plant and the need for improved processing and handling techniques to eliminate the potential risk associated with the observed high levels of cyanogenic glycosides.

Key words comparative, phytochemical, analysis, extracts, nicotiana tabacum, nigeria

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Introduction

Nicotiana tabacum, is an annually-grown herbaceous plant that is native to tropical and subtropical America, but it is now commercially cultivated worldwide. There are over 70 known species of *Nicotiana Tabacum* with wide variety in pigments and colours depending on environmental and soil conditions¹. However, all varieties require warm temperatures for effective growth and this is the reason why the plant thrives in Nigeria, where it is considered a prominent cultivated cash crop, especially in the Southern region [1].

Nicotiana tabacum is endowed with numerous primary and secondary bioactive metabolites which play essential roles in their adaptation to the environments, and their synthesis is often induced by different kinds of biotic and/or abiotic stresses [2]. The concentrations of phytochemical constituents in a plant are affected by environmental factors, cultivation methods and diseases [3].

It is also known that the types and concentrations of phytochemicals in tobacco leaves change during ripening, drying, fermentation, and storage period, and are influenced by a variety of factors such as processing methods, climate and temperature [4, 5, 6].

Hence, we postulate that substantial variation in phytochemical constituents may exist between foreign and local grown varieties of *Nicotiana tabacum* marketed in Southeastern Nigeria.

The specific objective of the study was to analyze and compare the phytochemical composition of dried leaf extracts of foreign and Nigerian locally grown varieties of *Nicotiana tabacum*.

Methods

Plant material

Two major sources of tobacco were identified during a preliminary survey of tobacco markets in Enugu metropolis. These were: A, Locally grown tobacco; and B, Foreign grown and processed tobacco leaf variety. The German Virginia tobacco (FCV German Leaf GmbH & Co. KG), which was selected for the study was among the more commonly used foreign tobacco. Representative single batches samples of local and foreign varieties of dry

tobacco leaves purchased from a major tobacco markets in Enugu metropolis were appropriately labeled for identification. The leaf samples were botanically authenticated at the Department of Botany University of Nigeria Nsukka, Nigeria. All the samples were analyzed for compositions, qualitative and quantitative phyto-chemistry.

Preparation of extracts

Dried leaf extracts of *Nicotian tabacum* were ground into coarse powder. The coarse powder was subjected to aqueous extraction in a soxhlet apparatus for 10 hours as follows: Fifty grams of the powdered leaves were placed in the inner thimble of the soxhlet extractor apparatus. Water was passed through the inner timble via a condenser with reflux system, from a round bottom flask containing 450ml of distilled water and placed on a thermostatic heating mantle regulated at 100 °C.

After filtration through Whatman filter paper No. 40, the filtrate was slowly evaporated to dryness on an electrothermal heating mantle regulated at 60 °C. The extracts were stored in screwed cap vials at 4-8°C until further use.

Phytochemical Analysis

Phytochemical analysis was done at Spring Board Research Laboratory Awka, Anambra state, Nigeria. Aqueous extract was evaluated for the presence of alkaloids, tannins, saponins, flavonoids and phenolic compounds using methods described by Zou et al [7], Kelley and Nelson [8].

Quantification by Gas chromatography equipped with a flame ionization detector (GC-FID).

The quantitative analysis of phytochemicals was performed on a BUCK M910 Gas chromatography equipped with a flame ionization detector [9]. A RESTEK 15 meter MXT-1 column (15m x 250um x 0.15um) was used. The injector temperature was 280 °C with splitless injection of 2ul of sample and a linear velocity of 30 cms⁻¹, Helium 5.0 pa.s was the carrier gas with a flow rate of 40 mlmin⁻¹. The oven operated initially at 200 °C, It was heated to 330 °C at a rate of 3°C min⁻¹ and was kept at this temperature for 5min. The detector operated at a temperature of 320 °C.

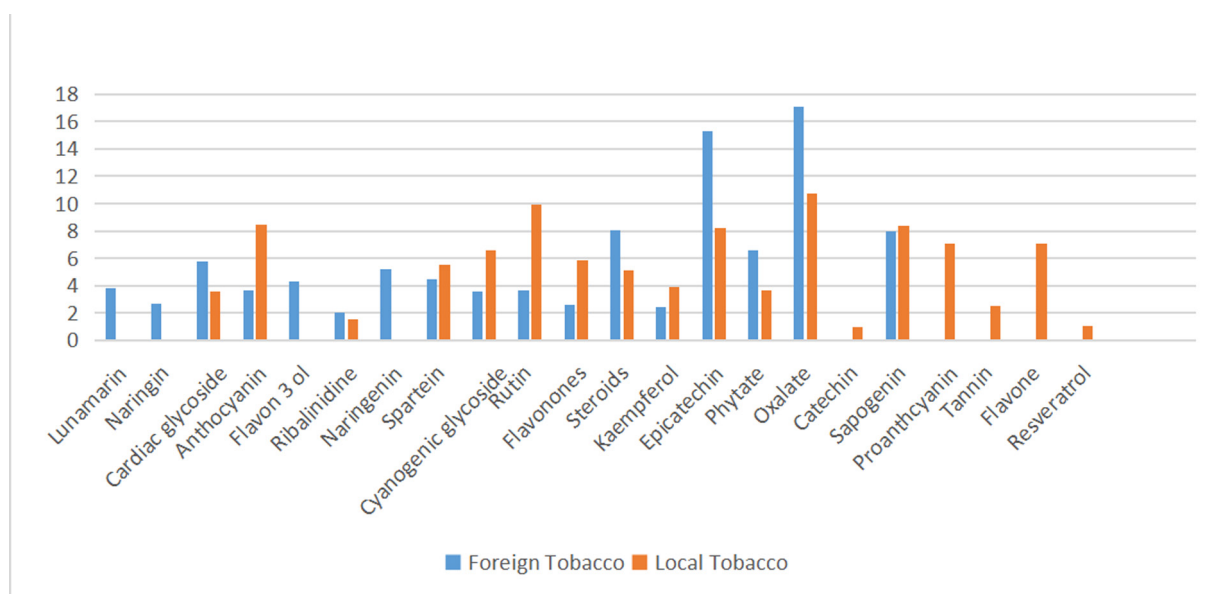


Figure 1. Percentage(relative) phytochemical composition of foreign and local tobacco extracts.

Collected: 29 October 2022
 Description: FID
 Column: RESTEK 15METER MXT-1
 Carrier: HELIUM AT 5PSI
 Data file: Phytochemical
 Sample: Foreign tobacco

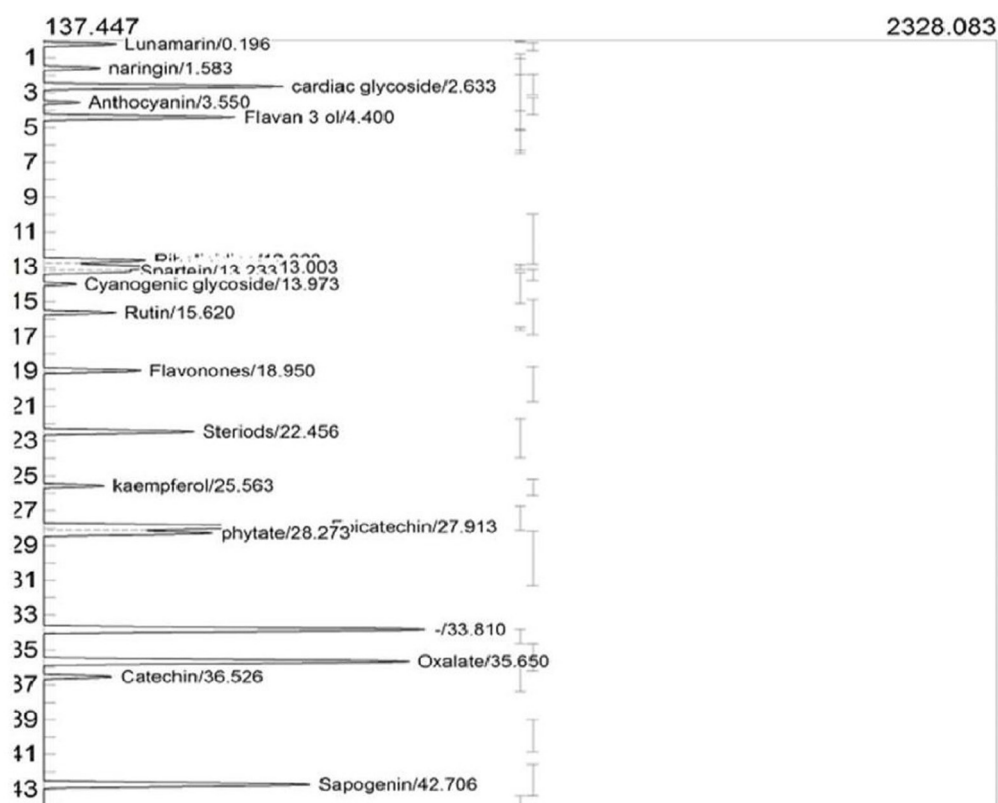


Figure 2. Plate 1-Column chromatography showing quantitative phytochemical analysis for aqueous foreign.

The quantification of non-volatile, high-molecular-weight, and thermally unstable compounds (such as Anthocyanins, Rutin, Tannins, Cardiac Glycosides, Cyanogenic Glycosides) was done using Gas Chromatography linked with a flame ionization detector (GC-FID) after applying a chemical derivatization process that converted them into more volatile, thermally stable, and easily detectable forms [9].

Phytochemicals were determined by the ratio between the area and mass of internal standard and the area of the identified phytochemicals. The concentration of the different phytochemicals was expressed in mg/kg.

Statistical analysis

Data were presented as means \pm standard deviation for continuous variables and as proportions for categorical variables. Comparison of continuous variables between the foreign and local tobacco products were made with independent Student's t-test. For discrete

variables distribution between groups were compared with Chi-square test and Fishers exact test as appropriate (where an expected cell is less than 5). All statistical analyses were carried out using the Statistical Packages for Social Sciences (SPSS Inc. Chicago Illinois) software version 25.0. Statistical tests with probability values less than 0.05 were considered statistically significant.

Results

The results of qualitative and quantitative phytochemical analysis of leaf extracts of foreign and locally grown tobacco are presented in Table 1 and Figure 1. While some constituents were well distributed in both tobacco varieties, significant differences were observed in phytochemical composition of the local and foreign tobacco leaf extracts. Compared to the foreign tobacco variety, the local *Nicotiana tabacum* leaf extract had higher absolute and relative concentration of Anthracyanin (10.18 ± 1.79 mg/kg; versus 4.99 ± 0.75 mg/kg), Cyanogenic glycoside (7.88 ± 1.64 mg/kg;

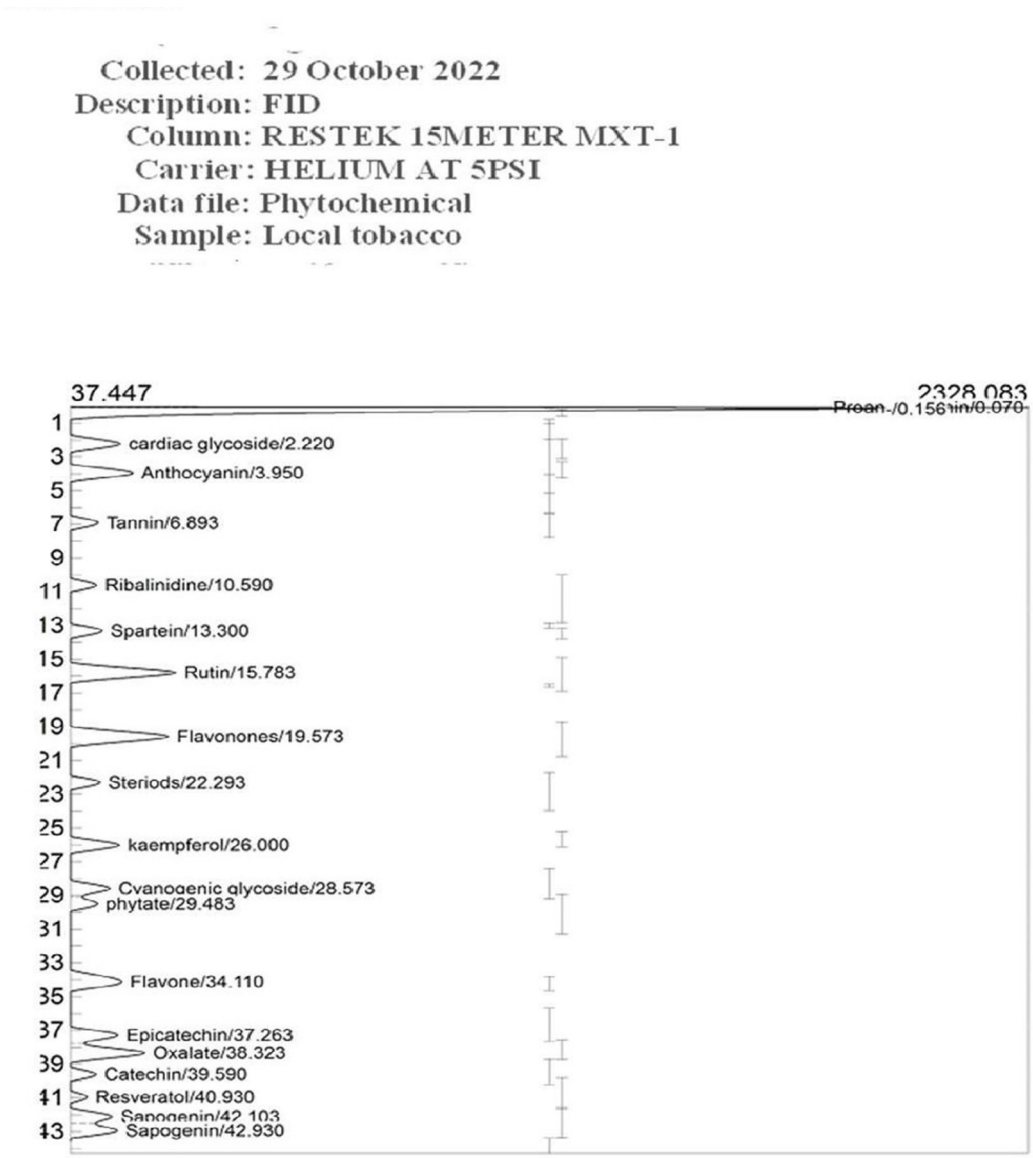


Figure 3. Plate 2-Column chromatography showing Quantitative phytochemical analysis for aqueous local tobacco leaf extract.

kg; 4.86 ± 0.43 mg/kg), Rutin (11.92 ± 1.65 ; 4.98 ± 0.87 mg/kg), and Flavonones (7.01 ± 0.76 mg/kg; 3.55 ± 0.41 mg/kg). On the other hand, the foreign tobacco was found to have greater concentration (mean \pm SD) of Cardiac glycoside, Steroids, Epicatechin, Phytate and Oxalate than the locally grown tobacco variety: (7.93 ± 0.59 ; 4.26 ± 0.88 mg/kg, 10.99 ± 1.85 ; 6.11 ± 0.38 mg/kg, 21.00 ± 2.18 ; 9.82 ± 1.20 mg/kg, 8.98 ± 1.50 ; 4.40 ± 0.54 mg/kg, and 23.49 ± 2.58 ; 12.94 ± 1.31 mg/kg, respectively for foreign and local tobacco leaf extracts; $p=0.0001$) (Table 1). Some components were however, exclusively specie specific for the foreign tobacco (Lunamarin, Naringin, Flavon-3-ol, Naringenin) and the local tobacco (Proanthocyanin, Tanin, Flavone, Resveratrol), being virtually undetected in the other species (Table 1). Similar trend was observed in the comparison of relative abundance of phytochemical compounds in the tobacco extracts as presented in Figure 1. Oxalates are the most abundant (in %) for both foreign and local extracts (17.06; 10.77), followed in decreasing order by Epicatechin (15.30; 8.19) and Sapogenin (9.93; 8.34).

The spatial distribution of the various phytochemical components on chromatographic column of the Gas chromatography equipped with a flame ionization detector (GC-FID) used for their quantification which is determined by the retention time, are displayed in Plates 1 and 2 (Figure 2 to 3).

Discussion

Phytochemicals are chemicals produced by plants through primary or secondary metabolism and generally have biological activity in the plant host and play a role in plant growth or defense against competitors, pathogens, or predators [10, 11].

Phytochemicals can be classified into major categories, such as alkaloids, carotenoids and polyphenols. Polyphenol compounds constitute a diverse group which include simple phenols, flavonoids, and non-flavonoids. Coumarins and Phenolic acids are sub-groups of simple phenols which also includes phytochemical sub-classes such as hydroxybenzoic acids, hydroxycinnamic

Table 1. Comparison of absolute concentration of phytochemicals in foreign and local tobacco extracts: A quantitative analysis.

Components	Foreign tobacco mg/kg (SD)	Local tobacco mg/kg (SD)	T-test	Significance
Proanthocyanin	ND	8.49 (1.67)	-	-
Lunamarin	5.26(0.13)	ND	-	-
Naringin	3.69(0.94)	ND	-	-
Cardiac glycoside	7.93(0.59)	4.26(0.88)	8.5178	0.0001*
Anthocyanin	4.99 (0.75)	10.18 (1.79)	6.5553	0.0001*
Flavon 3 ol	5.93 (0.88)	ND	-	-
Ribalinidine	2.75 (0.66)	1.86 (0.25)	3.1316	0.0106*
Naringenin	7.18 (0.95)	ND	-	-
Sparteine	6.11 (0.71)	6.61 (0.87)	1.0713	0.3091
Cyanogenic glycoside	4.86 (0.43)	7.88 (1.64)	4.3747	0.0014*
Rutin	4.98 (0.87)	11.92 (1.65)	9.1286	0.0001*
Flavonones	3.55 (0.41)	7.01 (0.76)	9.8085	0.0001*
Steroids	10.99(1.85)	6.11 (0.38)	6.3306	0.0001*
Kaempferol	3.38(0.49)	4.69 (0.35)	5.3788	0.00031*
Epicatechin	21.00 (2.18)	9.82(1.20)	11.0270	0.0001*
Phytate	8.98 (1.50)	4.40 (0.54)	7.030	0.0001*
Oxalate	23.49 (2.58)	12.94 (1.31)	8.8974	0.0001*
Catechin	1.35 (0.12)	1.13(0.37)	1.3689	0.201
Sapogenin	10.89 (1.18)	10.02 (1.27)	1.2285	0.2474
Proanthcyanin	ND	8.49(1.67)	-	-
Tannin	ND	3.00 (0.73)	-	-
Flavone	ND	8.49 (0.76)	-	-
Resveratrol	ND	1.29 (0.73)	-	-

*Statistically significant (independent T-test), ND= Not detectable.

acids, flavonols, flavones, iso-flavones, flavanols, flavanones, anthocyanins, chalcones. Flavanols are further classified as catechins, epicatechins, and proanthocyanidins [11, 12]. Tannins, lignans, and stilbenes are classified as non-flavonoids [11, 12].

Nicotiana tabacum is endowed with numerous bioactive metabolites which account for the rich phytochemical constituents identified in the tobacco leaf extracts in this study. A total of 22 phytochemicals were identified in tobacco extracts evaluated in this study. Major constituents found include; Oxalates, Epicatechin, Anthocyanin, Rutin, Sapogenins, Steroids and glycosides (cardiac and cyanogenic). Similar findings were reported by several studies within and outside Nigeria [13-16]. The abundance of phytoactive constituent such as Sapogenin, anthracyanin and Rutin is

responsible for the medicinal properties of *Nicotiana tabacum*. However, some adverse effects of the plant have been noted and attributable to the presence of toxic alkaloids [17].

A comparative phytochemical analysis of crude leaf extracts of locally grown and imported varieties of *Nicotiana tabacum* showed significant variations in concentration and types of constituents. The local *Nicotiana tabacum* leaf extract has higher abundance of Anthracyanin, Cyanogenic glycoside, Rutin, and Flavonones. These Poly-Phenolic phytochemicals are plant components with poly-hydroxyl groups, which are widely distributed in plants tissue and commonly used as antioxidants in the human diet due to their high reactive oxygen radical scavenging capacity [18, 19].

On the other hand, the foreign tobacco was found to have

greater content of Cardiac glycoside, Steroids, Epicatechin, Phytate and Oxalate. It is known that steroid hormones are produced in mammalian female and male gonads, and adrenal glands. However, reports of steroid production by plants are also been noted in several studies [20, 21]. Steroids present in the plant help its defense mechanism against certain viruses and pathogens. Previous studies have found that tobacco plants are capable of synthesizing animal steroid hormone such as progesterone and androst-4-ene-3, 17-dione, testosterone, and estradiol [20-22].

Some phytochemical components were however, exclusively specie specific for the foreign tobacco (Lunamarin, Naringin, Flavon-3-ol, Naringenin) and the local tobacco (Proanthcyanin, Tanin, Flavone, Resveratrol), and were virtually undetected in the other species. Tobacco leaves have been found to contain many kinds of polyphenols, among which phenolic acid and flavonoid glycosides constitute a large proportion [23, 24].

Germany is a leading source of imported raw tobacco in Nigeria and the main source of foreign tobacco in Enugu metropolis [25]. The obvious differences in geographical growth and post-harvest processing would account for the variation in phytochemistry found in this study between crude extracts of foreign and locally grown tobacco.

The concentrations of phytochemical constituents in a plant are affected by the environmental stresses such as drought, heat/cold, mineral deficiencies and diseases. They play essential roles in the adaptation of plants to their environments, and their synthesis is often induced by different kinds of stress conditions [1-3].

Several studies have shown that abiotic stress such as drought and salinity stress enhanced the total phenolic content, and flavonoid contents and individual phenolic acids and flavonoid compounds [1, 3]. The intensity of the antioxidant activities of these agents is based on their ability to combine with free radicals and their function is to protect plants from ultraviolet radiation, fungal pathogens, and avoid cells damaged by oxidative stress [26]. In addition, polyphenols also contribute to plant color and aroma attributes [1]. Phenolic compounds identified in our study samples include Flavon- 3- ol, Flavonones, Favones, Tanins, Kaempferols, Rutin, and anthocyanins. These were significantly more abundant in the local tobacco variety.

Saponins and Sparteine, a lupin alkaloid were found to be well distributed in all the extracts evaluated in this study. These phytochemicals are known to be increased in content when plants are subjected to water stress and stress saline conditions and composition may vary markedly depending on the surrounding environmental factors.

The significantly higher levels of cyanogenic glycosides in the local leaf extracts reflect an essential natural chemical defense mechanism of the plant to deter herbivores by releasing cyanide when plant tissues are damaged [27]. However, the high cyanogenic glycoside content in Nigerian tobacco products poses a serious health risk arising from the release of toxic hydrogen cyanide upon processing or smoking, and potentially causing acute poisoning or chronic neurological disorder. There is therefore a need for improved tobacco processing methods to reduce cyanogenic glycosides and lower health risks for tobacco farmers and consumers [27, 28].

Several studies have reported high cyanide levels in various other Nigerian staple foods such as cassava, sorghum, cocoyam, certain seeds/nuts (tiger nuts, cashew nuts) and certain leafy vegetables like bitter leaf [28, 29]. Traditional detoxification methods such as fermentation, drying, and cooking have been employed to reduce cyanide levels in natural food produce to safe limits for consumption [29].

The findings from analysis of the chemical composition of leaf extracts of *Nicotiana tabacum* evaluated in this study corroborate previous report of variations in the phytochemical composition

depending on plant genetics, growing conditions, processing technique and geographical location. The observed significantly high abundance of phytochemical constituents in the locally grown *Nicotiana tabacum* leaf extract in this study has far reaching implications especially considering the potential medicinal, industrial and economic benefits which can be harnessed and exploited.

However, the high levels cyanogenic glycosides in Nigerian tobacco leaf extracts may pose significant human health risks, requiring awareness, improved processing techniques, and greater regulatory attention to protect consumers and tobacco workers from cyanide toxicity.

Conclusions

The large number of phytochemical constituents identified in leaf extracts of *Nicotiana tabacum* indicates the potential medicinal value of this plant. Further studies are required to explore and exploit this potential for beneficial use.

Acknowledgments

No applicable.

Ethics approval

No applicable.

Data availability

The authors confirm that the data supporting the findings of this research work are available within the article. Any other data/information will also be available from the corresponding author on request.

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None received.

Authors' contribution

N.I.O was responsible for conceptualization, study design, data analysis and writing of manuscript; C.P.C and S.I.G shared in the study design and data analysis; N.I.N, O.C.N, K.I.N and C.C.O participated in data collection and data analysis. All authors shared in the final preparation and review of the manuscript.

Competing interests

The authors declare that they have no competing interests.

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