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Original Research Article

DETERMINATION OF PHYTOCHEMICALS AND MELATONIN CONTENT OF SELECTED FOOD STUFFS IN LAGOS, NIGERIA

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ABSTRACT

Nigeria's rich biodiversity includes many underutilized foods with potential nutraceutical benefits. This study investigated the melatonin content and phytochemical diversity of fifteen commonly consumed Nigerian plant-based foods to assess their potential for use in managing sleep disorders as alternatives to synthetic drug treatments. Samples of cassava, yam, sweet potato, corn, rice, beans, banana, watermelon, orange, pineapple, tomato, onion, garlic, mushroom and walnut were purchased from local markets in Lagos, Nigeria. The samples were prepared and subjected to solvent extraction using hexane:methanol: water (3:3:1). Standard qualitative phytochemical screening of the extract targeted seven bioactive classes of alkaloids, saponins, tannins, terpenoids, flavonoids, glycosides, and steroids. Melatonin levels of the samples were quantified using validated HPLC-DAD after clean-up with solid phase extraction cartridges and filtration using 0.22 µm PTFE syringe filters. The results of the phytochemical screening revealed substantial variability among the food samples, with cassava and orange containing all the phytochemicals tested. Melatonin content was found in the range of 2.62 – 64.31 ng/mL with pineapple having the highest concentration and watermelon, tomato, walnut, garlic and onion containing no detectable levels. These results indicate the nutraceutical potential of some Nigerian foods and the need for further investigation into bioavailability and health implications especially regarding sleep regulation.

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INTRODUCTION

Phytochemicals, the bioactive compounds naturally occurring in plants, have gained substantial scientific attention due to their diverse and profound health promoting properties [1]. These compounds, which include alkaloids, saponins, tannins, terpenoids, flavonoids, glycosides, and steroids exhibit significant biological activities such as antioxidant, anti-inflammatory, and anticancer effects [2]. The incorporation of phytochemical rich foods into daily diets is important for enhancing the body's defence mechanisms and reducing the risk of chronic illnesses, thereby contributing to improved health and longevity [3].

Recent years have seen a surge in research focused on elucidating the nutritional and bioactive profiles of commonly consumed foods, especially in regions where these foods serve as dietary staples [4]. This growing interest stems from the increasing recognition of their potential to mitigate chronic diseases such as diabetes, cardiovascular disorders, and obesity through their bioactive constituents [5]. Identifying the phytochemical and melatonin contents of these foods provides a scientific basis for dietary recommendations, ultimately fostering healthier eating habits [6].

On the other hand, the escalating reliance on synthetic drugs for managing conditions such as sleep disorders has raised

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concerns regarding their side effects and potential for abuse [7]. Natural alternatives like phytomelatonin present promising solutions due to their lower risk profiles and additional health benefits [8]. Melatonin, a critical regulator of the sleep wake cycle, is also recognized for its potent antioxidant properties [9]. Its presence in various foods, including fruits, vegetables, grains, and nuts, makes dietary sources of melatonin a natural alternative for improving sleep quality, enhancing immune function, and reducing oxidative stress [10]. However, the melatonin content in these foods is highly variable, influenced by species, environmental conditions, and processing methods [11].

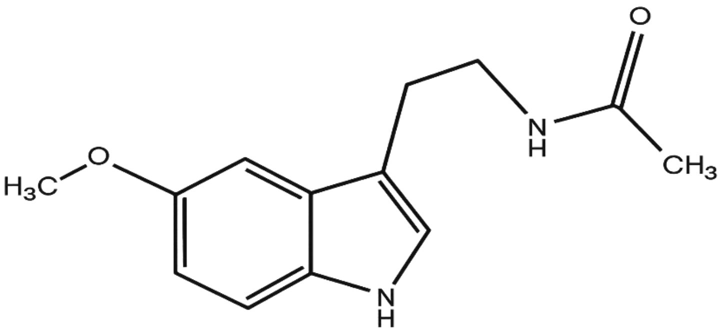

Figure 1: Chemical Structure of Melatonin.

Nigeria, with its rich agricultural diversity, offers a wide array of food resources, including fruits, vegetables, and cereals integral to the diets of its population. Despite this diversity, there is limited comprehensive data on the phytochemical composition and melatonin content of these foods [12]. This knowledge gap hinders a full understanding of their nutritional and therapeutic potential, which could otherwise guide public health strategies and agricultural policies.

High Performance Liquid Chromatography with Diode Array Detection (HPLC DAD) has emerged as a robust analytical tool for characterizing complex biological matrices [13]. This technique combines the high separation efficiency of HPLC with the spectral identification capabilities of DAD, making it ideal for analysing melatonin in food samples. HPLC operates by utilizing a high pressure mobile phase to transport analytes through a stationary phase, facilitating their separation based on molecular interactions such as adsorption, partitioning, or ion exchange. DAD further enhances this analysis by capturing absorbance spectra across a wide wavelength range (190–800 nm), enabling precise identification and quantification of eluted compounds [14, 15].

This study aims to investigate the phytochemical constituents and melatonin content of fifteen commonly consumed food types in Nigeria. By employing qualitative phytochemical tests and High Performance Liquid Chromatography with Diode Array Detection (HPLC DAD) for melatonin quantification, the research seeks to provide a comprehensive and reliable profile of these foodstuffs. The findings from this study are expected to contribute to the growing body of knowledge on the bioactive properties of Nigerian foodstuffs, with implications for nutrition, public health, and agricultural practices.

MATERIALS AND METHODS

Materials and Equipment

Samples of foodstuffs (cassava, yam, sweet potato, corn, rice, beans, banana, watermelon, orange, pineapple, tomato, onion, garlic, mushroom and walnut), HPLC DAD (Agilent 1260), amber vials (Berlin Packaging), centrifuge (Eppendorf 5804), analytical balance (Mettler Toledo), blender (Vitamix 5200), 24 mesh sieve (Retsch), Oasis® Hydrophilic Lipophilic Balanced Solid Phase Extraction Cartridges (Waters Corporation), Methanol HPLC Grade 99.9% (Thermo Fisher Scientific), Acetonitrile HPLC Grade, 99.9% (Thermo Fisher Scientific), Melatonin Standard 98% (Sigma Aldrich), Methanol 100% (Sigma Aldrich), Hexane 99% (Sigma Aldrich), Distilled Water.

Collection and Preparation of Samples

The selected food samples were purchased from local markets in Lagos, Nigeria. The samples were thoroughly washed with tap water to remove dirt and surface contaminants.

Sample preparation was done following the methods of by Fernández Pachón et al. [16], with slight modification. The outer peels of the samples except corn, rice, beans, mushroom and tomato were carefully removed. The edible portions of the samples were chopped into smaller pieces and oven dried at an ambient temperature of 40 °C for 48 hours. After drying, samples were each grinded with an electric grinder to achieve a uniform powder. The powdered materials were then passed through a 24-mesh sieve to ensure consistency in particle size.

Each powdered sample (100 g) was subjected to solvent extraction using 100 mL hexane:methanol:water mixture (3:3:1). The extracts were filtered, and the filtrates were placed in a fume hood for 24 hours to allow organic solvent evaporation. The aqueous residues obtained after evaporation were further filtered and stored for subsequent phytochemical screening.

Evaluation of the phytochemical Content

Each extract was subjected to phytochemical screening for alkaloids, saponins, tannins, terpenoids, flavonoids, glycosides, and steroids using standard procedures [17].

Sample Clean Up

Sample clean-up was carried out using the methods of Waters Corporation as outlined in its technical guide [18-19]. Each hexane:methanol:water mixture (3:3:1) extract was centrifuged at 40^{0}C for 15 minutes, filtered and then passed through Oasis HLB cartridges following SPE protocol of conditioning, loading, washing and elution. The solvent was evaporated under a gentle stream of nitrogen gas and the dry residues were reconstituted in 1 mL of water: acetonitrile (60:40) for HPLC DAD analysis.

Determination of Melatonin Content

Preparation of Standard Solutions

A stock solution of 100 µg/mL was prepared by dissolving 2 mg of standard melatonin in 20 mL of HPLC-grade methanol. Working standard solutions of 5 ng/mL, 10 ng/mL, 15 ng/mL, and 20 ng/mL were subsequently prepared by serial dilution of the stock solution using the same solvent. The solutions were filtered through a 0.22 µm PTFE syringe filter before HPLC-DAD analysis. The standard solutions were analysed using High Performance Liquid Chromatography with Diode Array Detection (HPLC DAD) and the results was used to construct calibration curve for quantitative determination of melatonin [20].

Chromatographic Conditions

A reversed-phase mode HPLC, consisting of a binary pump, an autosampler (ALS) injector, and a thermostatic column compartment with an online degasser, was used. Other conditions were as presented in Table 1
.

Table 1: Chromatographic Conditions for the Analysis of Melatonin

Software:	ChemiStation
Stationary phase:	Phenomenex Luna C18 (2) (250 x 4.6mm I.D, 5µm)
Mobile Phase A:	Water (60%v/v)
Mobile Phase B:	Acetonitrile (40%v/v)
Flow rate:	1.0mL/min
Injection volume:	20.000 µl
Column Temperature:	40 °C
Wavelength:	210nm

Analysis of Samples

Analysis was done using the validated method of Fernández et al. [21]. Each clean sample extract was analysed using HPLC DAD under the same conditions as for the standard melatonin.

Statistical Analysis

Data obtained were subjected to descriptive statistical analysis and values of melatonin content were reported as mean + standard deviation.

RESULTS

Phytochemical Analysis

The scientific names and food classes of the samples used are shown in Table 2. Phytochemical screening was carried out on the food samples, and the results are presented in Table 3.

Table 2: The Scientific Names and Food Classes of the Samples

S/N	Sample Name	Scientific Name	Food Category
1	Cassava	*Manihot esculenta Crantz*	Root
2	Yam	*Dioscorea alata L.*	Tuber
3	Sweet Potato	*Ipomoea batatas (L.) Lam.*	Tuberous root
4	Corn	*Zea mays saccharata Sturtev.*	Cereal grain (Vegetable when fresh)
5	Rice	*Oryza sativa L.*	Cereal grain
6	Beans	*Phaseolus vulgaris L*	Legume (Seed)
7	Banana	*Musa acuminata Colla*	Fruit
8	Watermelon	*Citrullus lanatus (Thunb.) Matsum. & Nakai*	Fruit
9	Orange	*Citrus sinensis (L.) Osbeck*	Fruit
10	Pineapple	*Ananas comosus (L.) Merr.*	Fruit
11	Tomato	*Solanum lycopersicum L.*	Fruit
12	Onion	*Allium cepa L.*	Bulb (Vegetable)
13	Garlic	*Allium sativum L.*	Bulb (Vegetable)
14	Mushroom	*Pleurotus tuber regium (Fr.) Sing.*	Fungi (Edible mushroom)
15	Walnut	*Tetracarpidium conophorum Müll.Arg.*	Nut (Seed)

Table 3: Phytochemical Content of the Food Samples

S/N	Sample Name	Alkaloids	Saponins	Tannins	Terpenoids	Flavonoids	Glycosides	Steroids
1	Cassava	+	+	+	+	+	+	+
2	Yam	+	+	+	-	+	+	+
3	Sweet Potato	+	-	+	-	+	+	+
4	Corn	-	-	+	+	+	-	+
5	Rice	+	-	+	-	+	+	+
6	Beans	-	+	+	+	+	+	+
7	Banana	+	-	+	+	+	+	+
8	Water melon	+	+	+	+	+	-	-
9	Orange	+	+	+	+	+	+	+
10	Pineapple	+	-	+	+	+	+	+
11	Tomato	+	+	+	+	+	+	-
12	Onion	-	+	+	-	+	-	-
13	Garlic	-	+	+	+	+	+	+
14	Mushroom	+	+	-	+	+	-	-
15	Walnut	-	-	+	+	-	-	+

Calibration Plot and Melatonin Content of the Food Samples

Different concentrations of melatonin standard were analysed, and the results were used to plot a calibration curve. Figure 2 shows the standard calibration curve while Figure 3 presents the chromatograms of the standards. The clean samples were analysed and the concentrations of melatonin calculated using the calibration curve equation as shown in Table 4.

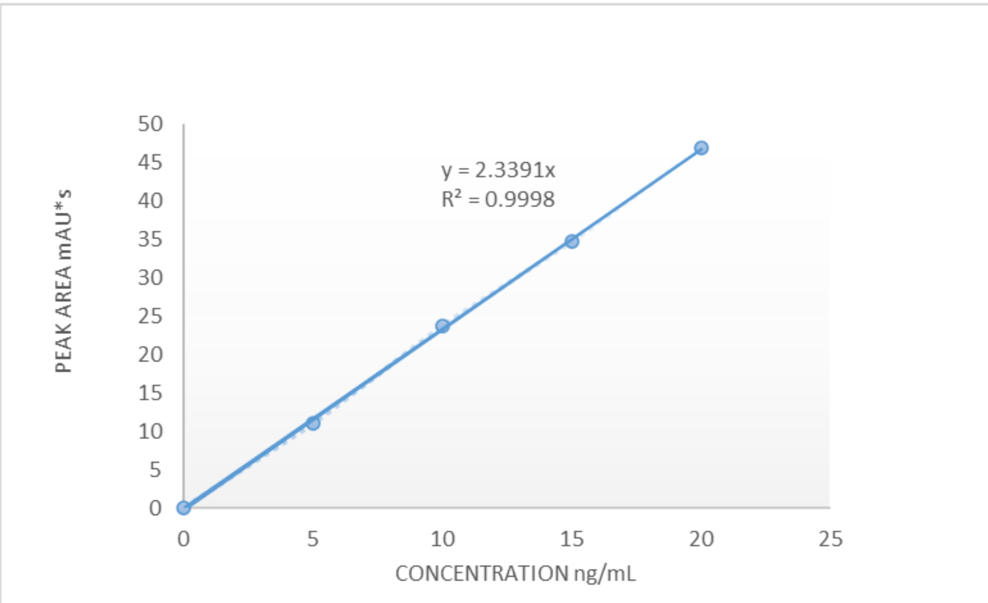

Figure 2: Calibration Curve for Melatonin Standard.

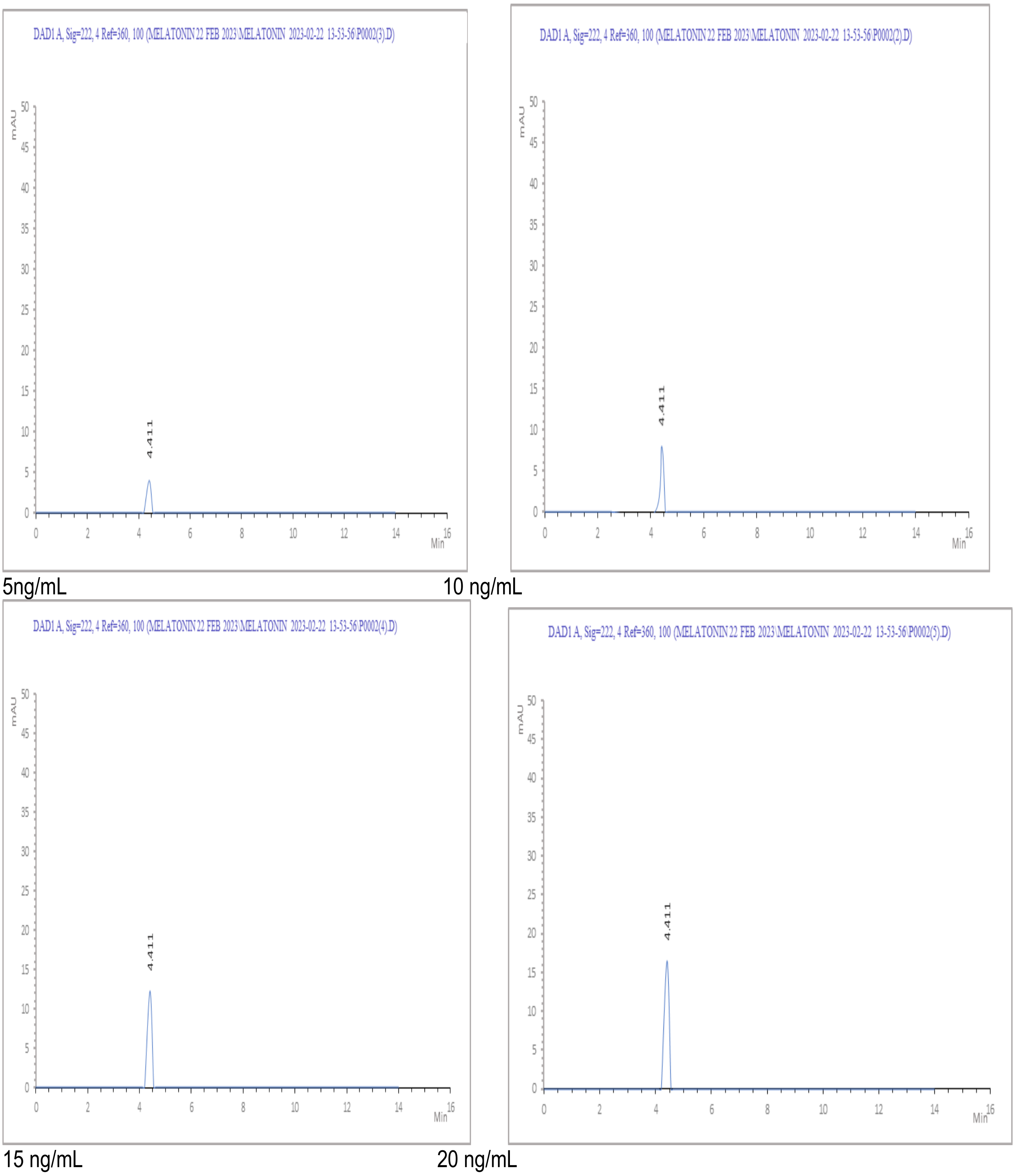

Figure 3: Chromatograms of Standard Melatonin at 5, 10, 15 and 20 ng/mL

Table 4. Concentrations of Melatonin in the Food Samples

S/N	Sample Name	Mean Peak Area (mAU*s)	*Concentrations of Melatonin (ng/mL)
1	Cassava	7.994	3.48 ± 18.60
2	Yam	14.000	6.09 ± 18.60

S/N	Sample Name	Mean Peak Area (mAU*s)	*Concentrations of Melatonin (ng/mL)
3	Sweet potato	21.355	9.28 ± 18.60
4	Corn	6.036	2.62 ± 18.60
5	Rice	22.420	9.75 ± 18.60
6	Beans	18.050	7.85 ± 18.60
7	Banana	8.254	3.59 ± 18.60
8	Watermelon	–	–
9	Orange	8.222	3.58 ± 18.60
10	Pineapple	147.911	64.31 ± 18.60
11	Tomato	–	–
12	Onion	–	–
13	Garlic	–	–
14	Mushroom	19.981	8.69 ± 18.60
15	Walnut	–	–
16	Standard	23.00	10.00 ± 18.60
17	Blank	–	–

*Concentrations in Mean ± SD

DISCUSSION

This study examined the phytochemical diversity of commonly consumed foods by screening for alkaloids, saponins, tannins, terpenoids, flavonoids, glycosides, and steroids. Cassava and orange samples exhibited a complete phytochemical profile, which aligns with previous reports documenting the broad phytochemical composition of these food items [22, 25]. All other samples contained phytochemicals ranging from 1 to 6 of the tested compounds. Previous studies have reported a range of phytochemicals including alkaloids, saponins, tannins, terpenoids, flavonoids, glycosides, and steroids in various parts of food plants such as roots, tubers, leaves, seeds, peels, and bulbs to identify bioactive compounds like [23, 29, 30, 35]. A relationship has been established between dietary phytochemicals and the nutritional and therapeutic properties of some plant-based foods [1, 4].

The melatonin concentrations in the various samples were in the range of 2.62 – 64.31 ng/mL, with pineapple exhibiting the highest concentration and corn the lowest. Other samples had variable amounts of melatonin. Previous studies have reported the presence of melatonin in sweet potato, mushroom, yam, orange and banana [36, 40, 41]. The presence of melatonin in these foodstuffs shows their potential health benefit, especially concerning sleep regulation.

Watermelon, tomato, onion, garlic, and walnut showed no detectable melatonin levels under the current analytical conditions. The observed variations in melatonin concentrations across the food samples maybe due to the influence of environmental conditions, cultivation practices, and post-harvest handling on melatonin accumulation in edible tissues. Factors such as light exposure, temperature, soil composition, and storage duration have been shown to significantly modulate melatonin accumulation and degradation in edible plant matrices [43].

The HPLC-DAD method used for the quantification of melatonin in the food samples proved reliable. It produced a linear calibration curve with a regression equation of $y = 2.3391x$ and a coefficient of determination (R^2) of 0.9994 signifying analytical accuracy, precision and sensitivity. This showed excellent linearity, indicating strong proportionality between concentration and detector response. All samples were analysed in triplicates and values presented in mean ± standard deviation to further ensure the precision and reproducibility of the measurements. HPLC-DAD has been used in several studies for the quantification of melatonin in foodstuffs and plant parts [21, 42, 43].

The phytochemical diversity and melatonin content of the food samples highlights their potential usefulness as nutraceuticals for sleep regulation.

CONCLUSION

This study shows a wide range of phytochemicals in the foodstuffs studied. The range of melatonin in the foodstuffs was 2.62 – 64.31 ng/mL, with pineapple having the highest concentration and corn the lowest concentration. The substantial phytochemical diversity and variable melatonin content of commonly consumed Nigerian foods demonstrate their potential significance as functional foods. Foods rich in multiple phytochemicals and melatonin, such as cassava, orange, beans, yams, and sweet potato, have potential for antioxidant, anti-inflammatory, as well as sleep regulatory properties.

The observed phytochemical diversity across these food samples demonstrates the broad potential of these foodstuffs as sources of health-promoting dietary supplements.

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AUTHORS CONTRIBUTIONS

EOA conducted the research and drafted the manuscript. COO supervised the research, provided intellectual guidance, and did a critical revision of the manuscript. SCA contributed to the study design and manuscript revision.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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