

Post Market Analysis of Some Neem Products Sold in Nigeria

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ABSTRACT

Context: The wide acceptability of herbal medicines as finished herbal products is hindered by the lack of quality control profile and total trust in the purity, safety efficacy and identity of the herbal product. **Aim:** The study evaluated the pharmacognostic, physicochemical, chromatographic properties and microbial load of commercially packaged neem products in Nigeria towards ascertaining its identity, content and purity. **Settings and Design:** Finished neem products were randomly purchased from different market sources and tested for compliance to already established standards documented by WHO, NHP, WAHP and USP. **Materials and Methods:** The macroscopic, microscopic, physicochemical and chromatographic properties of finished neem products were evaluated using standard procedures. Chemical-markers such as rutin and quercetin were quantified in the products using a validated HPLC method. Microbiological quality (bacterial and fungal counts) were assessed according to USP and WHO methods and guidelines. Descriptive statistics was used in the study. Results were represented in percentages and mean with respect to standard deviation where appropriate. **Results:** Macroscopic and microscopic evaluations showed the absence of adulterants and foreign matter and the presence of characteristic features of *Azadirachta indica*. Physico-chemical tests depicted varying results indicating differing sources of collection and possible soil types. **Conclusion:** The macro, microscopic, physicochemical, chemical and microbiological properties of neem products towards checks for identification and purity have been established. The neem products contained primarily *Azadirachta indica* and is considered safe for consumption. The results obtained are beneficial towards enriching current monographs of the neem products for commercialization.

Key words: Pharmacognostic, Microscopy, Microbial load, Validation, HPLC.

Key Messages: Market surveillance is essential to the proliferating use of herbal medicines. Good Agricultural, manufacturing, transportation and storage Practices must be encouraged and seen to be attainable. Finished herbal products must be well transported and stored in appropriate airtight packages. The absence of enteric pathogenic are central to consumption of quality herbal medicines.

INTRODUCTION

Herbal medicine use and users are increasingly more particular about the purity, safety, potency, and efficacy of medicinal plants as crude drugs which in-turn determine the eventual properties of the herbal medicinal product. Herbal medicines are still generally regarded as safe and cheap when compared to conventional medicines. The wild has been known to be the primary source of medicinal plants for locals and the herbal industry as large.^[1]

A major obstacle to the acceptability of herbal medicines is absence of quality control profile of raw materials and finished products. At the collection of raw material stage, the herbal medicine industry face challenges of adulteration and substitution and often fail microbial load assessment tests due to poor collection and storage practices as many raw materials are sourced from the wild and/or purchased from the market.^[2-4] The World Health Organization (WHO) lays emphasis on some basic parameters involving

morphological and phytochemical assessment of crude drugs towards the development of a quality control profile throughout the shelf life of the herbal product. For herbal medicines, attention is thus paid to quality indices as determined by organoleptic and microscopic evaluation, moisture and ash content; extractive values; qualitative and quantitative phytochemical evaluation through chromatographic studies and microbiological assessment.^[5-7]

Neem is the fresh or dried leaves of *Azadirachta indica* A. Juss (Meliaceae).^[8] Neem is largely sourced from the wild and consumed in Nigeria. All parts of the neem tree especially the leaves have been exploited commercially due to their medicinal properties.^[9] Reports on its effectiveness as an antibacterial, antiviral,^[10,11] immune boosting,^[12] anti-inflammatory,^[13] antioxidant,^[14] and anticarcinogenic,^[15] agent

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have been well documented. Due to its extensive use especially in the management of fever and viral infections, it is sometimes adulterated with other morphological parts and leaves of *Melia azedarach* (Meliaceae).^[16] Herbal products are sometimes found to have indicator pathogenic organisms.^[17] Hence, the study was aimed at assessing the pharmacognostic, physicochemical, chemical properties and microbial load of commercially packaged neem products in Nigeria towards ascertaining its identity, content and purity.

MATERIALS AND METHODS

Materials

Rutin and Quercetin reference standards (sigma). All solvents used for HPLC analysis were HPLC grade (Merck). 0.45µm Millipore membrane filter, amber coloured sample vials. All chemicals and reagents used for other analysis were of analytical grade.

Equipment

The chromatographic system used in this study was Shimadzu Ultra- Fast HPLC system (LC-20AB) equipped with Auto-Sampler (SIL- 20AC); column oven (CTO-20AC); Degasser (DGU-20A3); column (VP-ODS 5µm, 150 x 4.6 mm); Diode Array Detector (SPD-M20A); System Controller (CBM- 20Alite) and Windows LC solution software (Shimadzu Corporation, Kyoto Japan), Analytical weighing balance (Ohaus).

Methods

Sample Collection

Five (5) finished herbal commercial brands of neem samples coded FG, RG, HC and JUL were purchased and pooled from different market sources along with NN (a newly developed product in NIPRD) in Nigeria.

Pharmacognostic evaluation

Macro and Microscopy of Powdered Leaf Samples

Macroscopic studies/ organoleptic characteristics such as colour/ appearance, odour and taste were carried out on the powder samples. Each powder sample (5g) was macerated in 50 mL sodium hypochlorite TS until sufficiently cleared, washed with distilled water three times and then mounted with glycerol/ethanol TS.^[18] The presence of some metabolites were tested through chemo-microscopy studies of the samples. Reagents such as iodine, concentrated sulfuric acid, concentrated hydrochloric acid, ferric chloride, Sudan III, ruthenium red and phloroglucinol with conc. HCl (1:1) were used.^[19]

Physicochemical properties

Moisture content, total, sulphated, water soluble and acid insoluble ash content and water and alcohol extractive values were determined following WHO guidelines.^[18]

HPLC Analysis of Neem Products

Preparation of Sample Solution

Neem leaf powder (70 g) was extracted with ethanol (70%) by soxhlet extraction and concentrated *in vacuo*. The sample extract (1 mg) was weighed accurately and dissolved in 7 mL of acetonitrile in a 10 mL volumetric flask and made up to mark with acetonitrile. One (1) mL was then withdrawn into a 100 mL volumetric flask and made up to mark with acetonitrile.^[20] An aliquot of each sample was taken with withdrawn with a 2 mL syringe and filtered through a millipore membrane filter (0.45µm) into already labelled sample vials from which each sample was injected into the HPLC machine.

Assessment for Rutin and Quercetin

The mobile phase includes solvent A: 0.1% v/v phosphoric acid in water and solvent B: acetonitrile. Isocratic mode was used with injection volume of 10 µL; DAD detection was at UV 360 nm wavelength. The operating conditions of the HPLC machine was set at flow rate 1 mL/min of solvent B (20%) with a column temperature of 40°C and a run time of 20 min.

Each sample was thoroughly mixed and with a 2 ml syringe, an aliquot of each sample was withdrawn and filtered through a millipore membrane filter (0.45 µm) into labelled sample vials. The samples were then injected into the HPLC machine.

HPLC Method Validation for Quercetin and Rutin

Linearity, precision and accuracy according to ICH guidelines,^[21] was used to validated the HPLC method. Results of the analysis of different concentrations of mixtures of the standard was used to plot a calibration curve. The linear regression model was used to determine the correlation coefficient. The precisions of peak area measurements for the reference compounds were calculated as the relative standard deviations (RSD) of six repeated runs for intraday and three repeated runs for inter day precisions. The accuracy of the method was determined by carrying out HPLC analysis of three known concentrations of a mixture of quercetin and rutin. Each analysis was done in triplicates on the same day. The corresponding amount of the injected compounds was calculated from the calibration curve, and the recovery (%) of each analyte was calculated in relation to known concentrations.

Microbial Load Determination

Using aseptic techniques, 1g of each powdered sample was weighed into separate sterile Tryptic Soy broths (10 mL). Each sample (1 mL) was taken using a sterile pipette and dissolved in 9 mL sterile Tryptic soy broth and the mixtures were diluted 10-fold down to 10⁻⁴. Each of the 10⁻⁴ dilutions (1 mL) was directly inoculated into a sterile molten Tryptic Soy Agar (KASVI®) for Total aerobic bacterial count and Sabouraud Dextrose Agar (KASVI®) for Total fungal count. All microbial analyses were carried out in duplicates. The plates were allowed to solidify, dried and incubated at 37°C for 24-48 hr for bacterial growth and 25°C for 48-72 hr for fungal screening. After incubation, colony forming units were recorded from a colony counter. The microbial content was taken as the mean of duplicate determinations. Also, 1 mL of the 10⁻⁴ dilutions of each sample were plated onto selective media which includes Mannitol salt agar, Eosine methylene blue agar, Cetrimide agar, Salmonella Shigella agar and MacConkey agar. The plates were allowed to solidify, dried and incubated at 37°C for 24-48 hr for growth. The identification of the microbial colonies was done by observation of colony color, size, appearance, gram staining and cell morphology. For aerobic bacteria, samples that showed bacterial growth of more than 10⁵ in 1 g of herbal medicine was considered unsatisfactory according to WHO guidelines.^[22]

DISCUSSION

Neem is the fresh or dried leaf of *Azadirachta indica* A. Juss (Meliaceae).^[8] Neem products comprising of dried and comminuted leaf samples were studied and analysed based on label claim and content. The degree to which each product complied with labelling information as expected by National Agency for Food, Drug, Administration and Control (NAFDAC), the regulatory body for food and drugs in Nigeria was documented (Table 1). None of the products had all the information as expected. This calls for increased regulatory checks and oversight.

Results of organoleptic analysis showed similarities in taste and odour across all products studied. All products showed bitter taste and smell

Table 1: Documentation on Neem products.

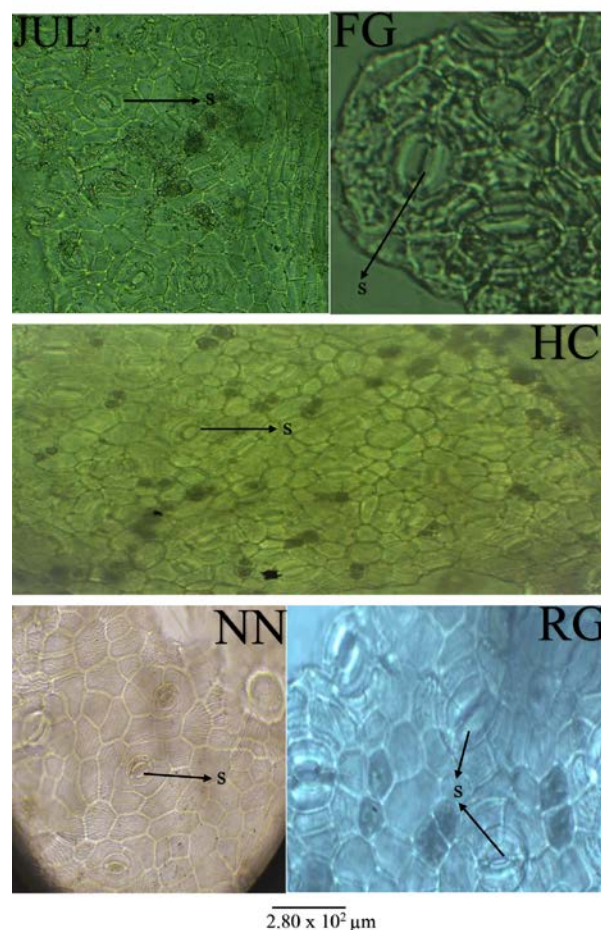
Sample Code	Presentation	MD	ED	Batch/Lot	Indication	Direction of use	NAFDAC number	Address of Manufacturer
FG	powder	no	yes	yes	yes	yes	Yes	No
RG	powder	yes	yes	no	yes	yes	no	No
HC	powder	no	yes	no	no	no	no	No
JUL	powder	no	yes	no	yes	yes	no	No
NN	powder	yes	yes	yes	yes	yes	no	yes

*MD- manufacturing date; ED- expiry date

Table 2: Organoleptic properties of Neem Products.

Samples	Taste	Colour	Odour	Particle Size (N)
FG		Light green		150 - 180
RG		Brownish green	Characteristic	150 - 200
HC	Bitter	Deep green	Odour	150 - 180
JUL		Brownish green		150 - 200
NN		Deep green		150 - 200

characteristic of *A. indica*. Whole neem leaves are light green in colour. The colour across all samples is green as characteristic of neem (Table 2) however the products varied slightly in shades of green. FG was light green whereas, RG and JUL were dark green while HC and NN were deep green shade. FG which had the finest particles was observed light green. The NN sample, was deep green in colour and was air dried under shade for 7 days. Improper/prolonged drying conditions wherein some leaves are rotting and brownish in colour before comminution or oven drying under high temperatures (higher than 40°C) might lead to variations in colouration of different batches. Particle size, method and period of drying can result in different colour shades of comminuted herbs.^[23] As shown in Figure 1, all products showed the presence of features that are characteristic of *A. indica* such as the presence of anomocytic, hypostomatic, polygonal epidermal cells with almost straight cell walls and the presence of metabolites such as oil, calcium oxalate crystals (Table 3). Stomata cells were observed primarily on one surface as expected and reported by West Africa Herbal Pharmacopoeia.^[8] Features such as fibres, crystal sands, polyhydric and rosette crystals were also observed across all samples. These features are characteristic of the powder microscopy of neem. West Africa Herbal Pharmacopoeia,^[8] reports that rosette crystals are present in the mesophyll which is interrupted by collenchyma in the mid region. Crystals were observed to be arranged and chiefly present across the mid rib section and within the fibres (Figure 2 and 3). Though trichomes have been reported to be occasionally present by the West Africa Herbal Pharmacopoeia (WAHP),^[8] and not reported at all by Nigerian Herbal Pharmacopoeia (NHP),^[24] a scanty presence of trichomes were observed in all samples except NN and JUL (Figure 4). Methods of drying such as oven, dehumidification and air drying methods have not been reported to cause damage to trichomes. The presence of trichomes is also dependent stage of maturity as they are formed from differentiation of a pool of equivalent cells as controlled by phytohormones and cytokinins which influence increased production of trichomes. A rapid decline in the density of trichomes as leaves age have been reported in some plant species.^[25,26] In some unpublished data by the authors, microscopy of epidermal surfaces of young whole leaves of neem revealed the scanty presence of multicellular trichomes.

Microscopy of Neem Products**Figure 1:** Stomata (s) characteristic of *Azadirachta indica* across all powder samples.

Key: FG; HC; JUL; RG; NN (neem products)

Table 3: Chemo-microscopic properties of Neem products.

Parameter	FG	RG	HC	JUL	NN
Cellulose	+	+	+	+	+
Mucilage	-	-	-	-	-
Starch	-	-	-	-	-
Lignin	+	+	+	+	+
Proteins	+	+	+	+	+
Oil	+	+	+	+	+
Tannin	+	+	+	+	+
Calcium oxalate	+	+	+	+	+

Key: + = present; - = absent

This differs from the unicellular trichomes observed in this study and on some mature, older leaves. A more extensive study on the trichomal pattern presence in neem leaves is needed.

Results of the physicochemical tests (Table 4) are compared to limits as stated by the monographs of *Azadirachta indica* in the WAHP,^[8] and NHP.^[24] Both pharmacopoeias reported the similar limits for the physicochemical parameters. Comparing results obtained with the limits stated by WAHP, moisture content limit - NMT 30 %, the

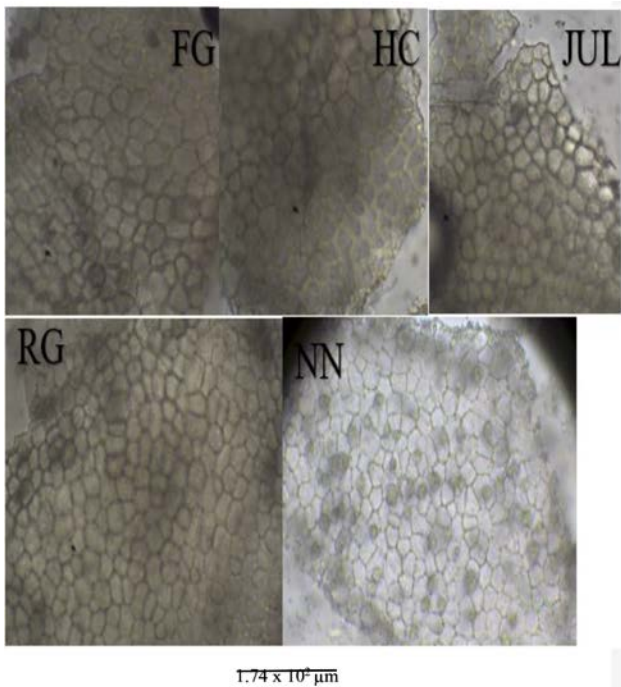


Figure 2: Epidermal cell pattern with no stomata characteristic of *Azadirachta indica* across all powder samples.
Key: Neem products - FG; HC; JUL; RG; NN.

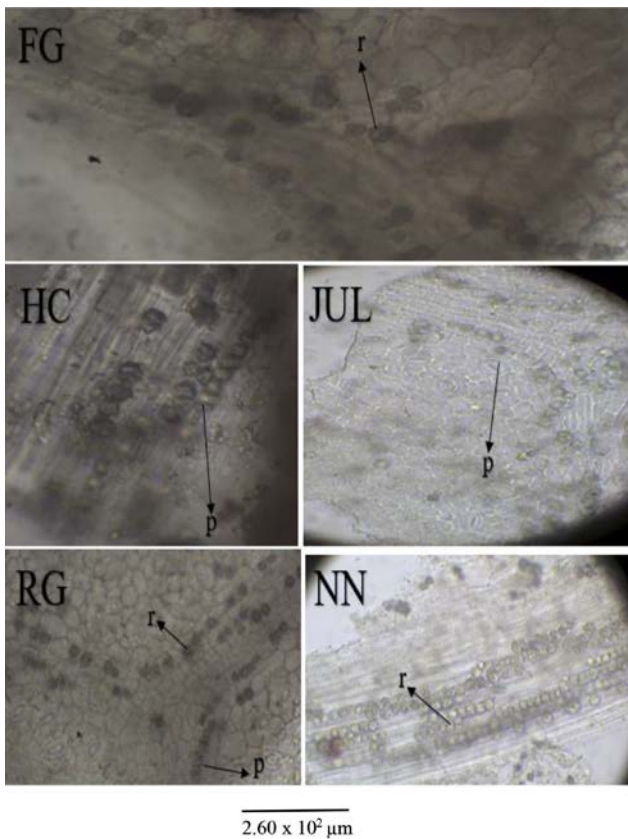


Figure 3: Arrangement of rosette (r) and polyhydric (p) druses crystal characteristic of *Azadirachta indica* across powder samples.
Key: FG; HC; JUL; RG; NN (neem products).

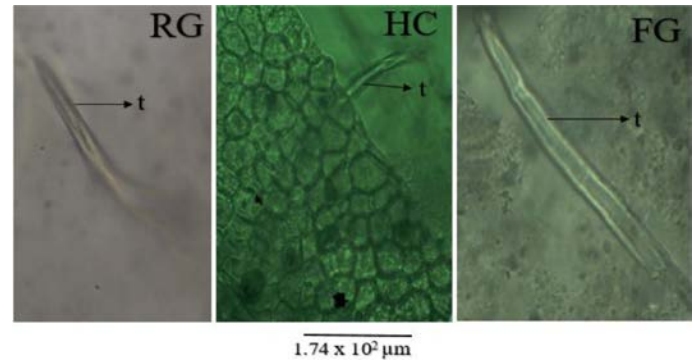


Figure 4: Trichomes (t) of *Azadirachta indica* across found in RG, HC and FG samples.

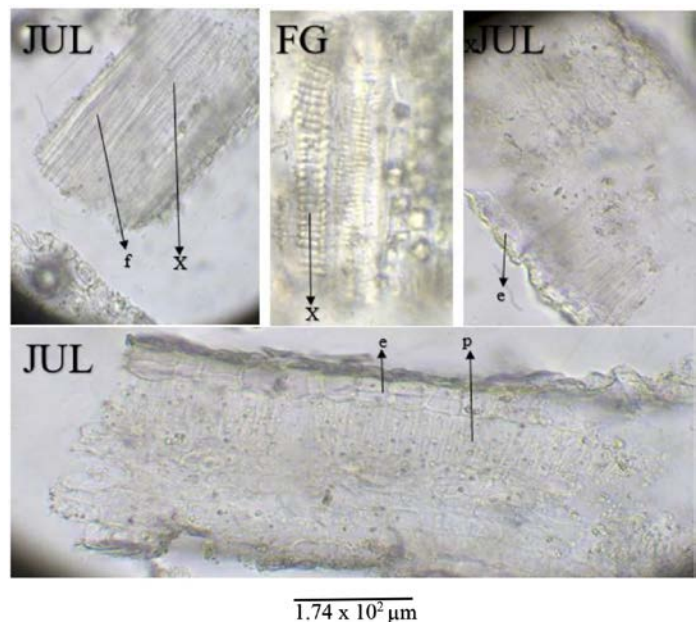


Figure 4: Sections of the midrib observed across FG and JUL powder samples.
Key: e- epidermal cells; f- fibre; p-palisade; x-xylem vessels

Table 4: Physicochemical Properties of Neem Products.

Sample code	Moisture content (%w/w)	Total Ash (%w/w)	Sulphated Ash (%w/w) n=3	Water soluble Extractive value (%w/w) n=3	Alcohol soluble Extractive value (%w/w) n=3
FG	7.4 ± 0.4	9.2 ± 0.2	11.0 ± 0.3	24.5 ± 0.4	19.6 ± 0.5
RG	9.5 ± 0	8.9 ± 0.4	4.4 ± 0.1	25.2 ± 0.2	20.1 ± 0.6
HC	9.3 ± 0.3	9.6 ± 0.2	14.2 ± 0.2	32.9 ± 0.6	23.9 ± 0.9
JUL	7.3 ± 0.3	8.6 ± 0.1	14.8 ± 0.1	25.4 ± 0.5	15.2 ± 0.3
NN	9.4 ± 0.5	8.8 ± 0.4	12.8 ± 0.4	29.8 ± 0.9	17.9 ± 0.1

HPLC Analysis of Neem Products

following results were obtained for the samples: FG - (7.4 ± 0.4) % w/w; RG - (9.5 ± 0) %w/w; HC - (9.3 ± 0.3) %w/w; JUL - (7.3 ± 0.3) %w/w; NN - (9.4 ± 0.5) %w/w (Table 2). Limit for total ash as stated by WAHP is NMT 11.6 % whilst the following results were obtained: FG - (9.2 ± 0.2) % w/w; RG - (8.9 ± 0.4) %w/w; HC - (9.6 ± 0.2) %w/w; JUL - (8.6 ± 0.1) %w/w and NN - (8.8 ± 0.4) %w/w. Limit for water soluble extractive as stated by WAHP is NLT 16 % whilst the following results were obtained: FG - (24.5 ± 0.4) % w/w; RG - (25.2 ± 0.2) %w/w; HC - (32.9 ± 0.6) %w/w; JUL - (25.4 ± 0.5) %w/w; NN - (29.8 ± 0.9) %w/w. Results for moisture content, total ash, water soluble extractive were observed to be within limits as stated by WAHP. However, the results obtained for alcohol soluble extractive of some samples fell out of specification, (WAHP limit-NLT 22.0 %w/w). The following obtained for alcohol soluble extractives were lower: - FG - (19.6 ± 0.5) % w/w; RG - (20.1 ± 0.6) %w/w; JUL - (15.2 ± 0.3) %w/w; NN - (17.9 ± 0.1) %w/w. Only HC - (23.9 ± 0.9) %w/w was observed to be within this specification. The results (Table 3) obtained for acid in-soluble ash and water-soluble extractive of some samples fell out of specification. Limit for acid-insoluble ash as stated by WAHP is NMT 1.2 %. Results of acid-insoluble ash obtained include: NN - (0.45 ± 0.2) %w/w; JUL (0.825 ± 0.4) %w/w; HC (1.03 ± 0.2) %w/w; RG (1.05 ± 0.07) %w/w. FG (1.85 ± 0.2) %w/w fell out of specification. While Limit for water-soluble ash as stated by WAHP is NLT 1.8 %. The following samples, RG - (1.825 ± 0.2) %w/w. NN - (1.075 ± 0.2) %w/w and HC (1.426 ± 0.2) %w/w had lower results than stated hence were out of specification while FG - (2.675 ± 0.4) %w/w; JUL - (2.7 ± 0.6) %w/w were within specification (Table 3). Physicochemical properties are a function of various factors such as particle size and more intrinsically, geographical location/collection site and seasonal variation/time of collection.^[27] These factors could cause slight differences, as observed between one product and the other. Values outside the limit for total ash would have depicted high inorganic content, showing the possibility that samples were collected from sites close to the road or industrial areas where the plant has been exposed to high inorganic material and further tests for heavy metal would be needed to ascertain safety levels for heavy metals. Efforts to enrich the soil with water soluble inorganic salts which will enhance yield of extractives and secondary metabolites should be made to maintain extractive values within limits. It is also important to maintain Good Agricultural Practices example is preventing over use of land. Extractives show how rich in either water or alcohol soluble constituents each batch collected is. Across all samples, on macroscopic and microscopic examination, no contaminants, foreign matter nor adulterants were observed showing evidence of good collection and manufacturing practices. Manufacturers are advised to follow good collection practices in order to ensure that each batch of plant material meets standard specifications which would ensure that bioactive constituents are in the maximum quantities possible.

In this study, rutin and quercetin were identified and quantified in all the brands of neem products with the aid of a HPLC (Figure 5-10). The

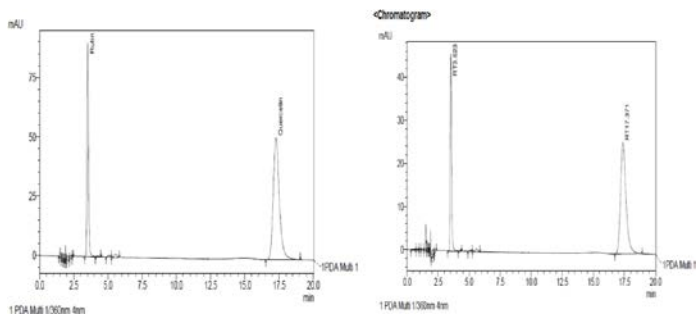


Figure 5: Chromatogram of Rutin (3.523 mins) and Quercetin (17.371 mins) reference standards.

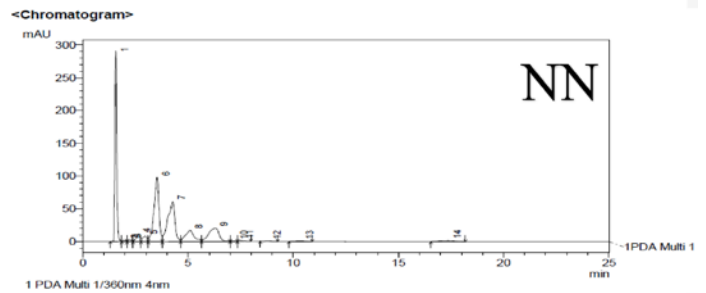


Figure 6: Chromatogram of Rutin (6) and Quercetin (14) in NN.

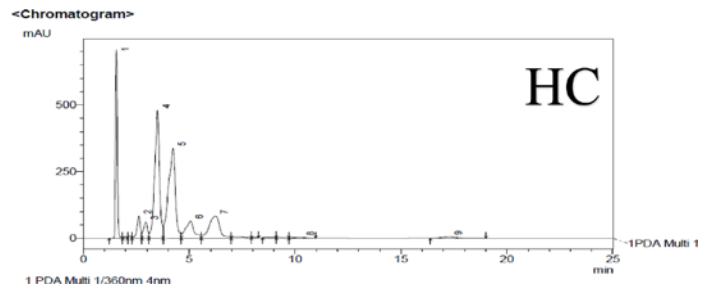


Figure 7: Chromatogram of Rutin (4) and Quercetin (9) in HC.

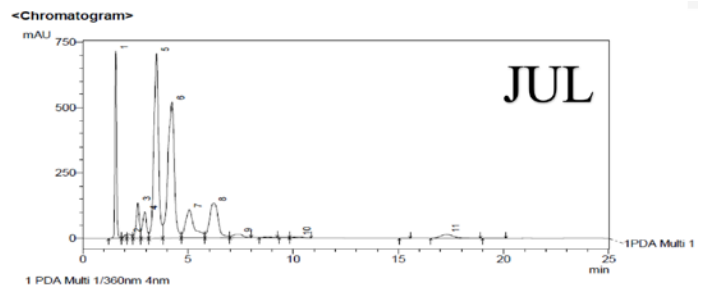


Figure 8: Chromatogram of Rutin (5) and Quercetin (11) in JUL.

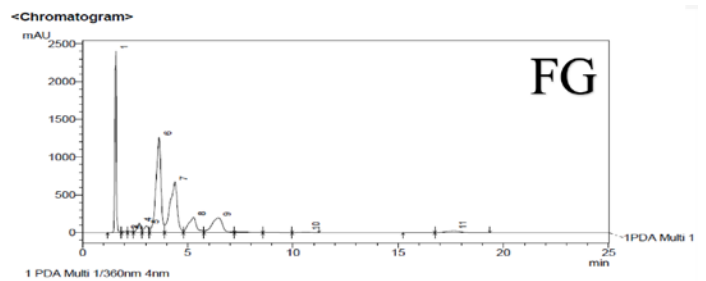


Figure 9: Chromatogram of Rutin (6) and Quercetin (11) in FG.

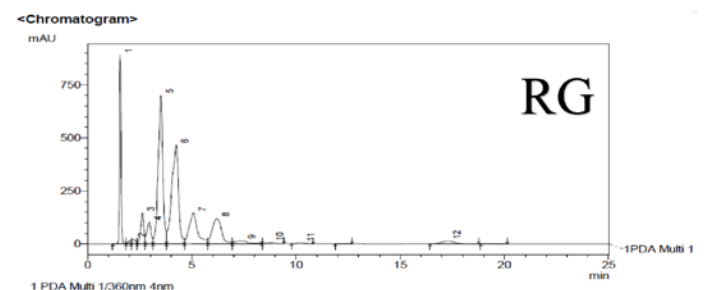


Figure 10: Chromatogram of Rutin (5) and Quercetin (12) in RG.

method used for the quantification of rutin and quercetin was validated and found suitable (Table 5). The LOD and LOQ of rutin were found to be 1.79 µg/mL and 5.98 µg/mL respectively while that of quercetin were 0.20 µg/mL and 0.66 µg/mL respectively (Table 6). The calibration curve of the two compounds showed good linearity with the correlation coefficient 0.999. The inter and intra-day precisions had a relative standard deviation (%RSD) less than 2. The method was accurate and the percentage recoveries of rutin and quercetin were 100.4% and 97.9% respectively (Table 6). Hence, it can be inferred that the method used in this work is adequate, accurate and precise for the quantitative analysis of rutin and quercetin in the extracts of neem products. From Table 6, amount of rutin in the samples ranged from 90.8 µg/mL to 1187.6 µg/mL while quercetin ranged from 4.6 µg/mL to 32.6 µg/mL in all the samples analyzed. More rutin was observed than quercetin across all samples. Previous studies by Vergallo *et al.*^[28] and Aarti *et al.*^[29] have confirmed the presence of rutin and quercetin in hydro-alcoholic extract of *A. indica*. Previous researchers have confirmed the presence of rutin and quercetin in neem leaves extract. Garima *et al.*^[30] developed the fingerprint profile of 50% ethanol extract of *Azadirachta indica* leaves with the aid of HPTLC, and revealed the presence of rutin, quercetin and other polyphenolic compounds as marker compounds of *Azadirachta indica* leaves. In another work, Pratima *et al.*^[31] isolated and further identified quercetin-3-O-β-D-glucoside, as a marker compound from aqueous extract of leaves of *Azadirachta indica*. Anima *et al.*^[32] observed that aqueous neem leaf extract contains 6 to 48 mg of quercetin and inferred that quercetin is one of the major bioactive flavonoids found in neem leaf extract. This justifies the choice of quercetin and rutin as marker compounds for the development of fingerprint pattern of the neem tea used in our study. In this study, as can be found in Table 5, rutin content in the samples have a range of 90.8 µg/mL to 1187.6 µg/mL and quercetin ranged from 4.6 µg/mL to 32.6 µg/mL. It is noted that the content of rutin in the samples is more than that of quercetin. This could be attributed to the solvent used for extraction. Vergallo *et al.*^[28] reported that the content of rutin is

more in hydro-alcoholic extract of *A. indica*. Similarly, Anokwuru *et al.*^[33] remarked that the amount of flavonoids in *A. indica* dependent on the solvent used for extraction. It is noteworthy that in this study, NN tea showed undetectable amounts of quercetin which is in agreement with the finding of Vergallo *et al.*^[28] Herbal teas have increasingly attracted a great deal of interest as they are widely believed to be a sources of potent antioxidant agents. In addition to the antioxidant properties, rutin and quercetin offer various therapeutic benefits such as antimicrobial, anti-inflammatory, anticancer, and hepatoprotective activities.^[34] This study has shown that Neem tea are rich in flavonoid compounds. In addition to the antioxidant properties, rutin and quercetin offer various therapeutic benefits such as antimicrobial, anti-inflammatory, anticancer, and hepatoprotective activities.^[34]

The antimicrobial assessment and quantification of bacteria and fungi that are able to grow aerobically in 1g or 1 mL sample revealed that none of the samples recorded microbial growth on SDA for total fungal count after 72 hr of incubation. The total aerobic bacterial plate count ranged from 4.0×10^4 cfu/mL - 7.0×10^4 cfu/mL. The least in total number of microbes present in RG (4.0×10^4 cfu/mL) while HC had a total of 7.0×10^4 . The highest was recorded with FG (Table 7). All samples were within acceptable limit of 10^4 cfu/mL according to United States Pharmacopoeia (USP),^[35] and 10^5 cfu/mL according to WHO,^[22] except for FG which was too numerous to count. Bacterial and fungal contamination are often encountered, particularly in herbal products sold in open markets and unpackaged herbal medicines. This is often due to lack of control of moisture and improper means of transportation and storage.^[22] All the neem products studied had moisture less than 12% therefore accounting for the low numbers encountered in bacterial count. Despite FG having a moisture content of 7.4%, total aerobic count indicates bacterial load was too numerous to count but no fungal count. Other reasons besides moisture could be responsible for the high bacterial load such as contaminated raw material. *Bacillus* species were isolated on TSA for all samples which are of health importance. The presence of *Bacillus* species may be as a result of improper handling of samples and contaminated processing equipment.^[36] Also, all the samples revealed the absence of indicator pathogenic microorganisms on selective media, thus indicating proper hygiene conditions during preparation and storage and possibly no risk in consumption. These results can be expected as all products were well packaged and properly sealed in airtight packages. Powdered products also often have less microbial contamination than liquid preparations due to the presence and quality of water used during preparation which could contribute to a high level of contamination.^[37]

No similar study has been directly reported on neem products, however a study carried out by Idu *et al.*^[38] on microbial load of some herbal medicinal products sold in some local open markets in Abeokuta, Nigeria revealed the presence of bacteria species ranging from 1.3×10^5 cfu/g to

Table 5: Method Validation.

Parameters	Rutin	Quercetin
Retention time (min) mean ± SD	3.51 ± 0.004	17.33 ± 0.01
Linear equation	Y=17306x + 42778	Y=38453x + 89792
Correlation coefficient (R ²)	0.9993	0.9995
Linear range (µg/mL)	2.34 - 300	2.34 - 300
Precision (Repeatability)- Inter day % RSD (n=6)	1.652361	1.21
Precision (Repeatability)- Intra-day % RSD (n=3)	1.193004	0.60
Accuracy (% Recovery)- Mean ± SD (n=3)	100.36 ± 0.77	97.93 ± 2.40
Limit of Detection (LOD) (µg/mL)	1.79	0.20
Limit of Quantification (LOQ) (µg/mL)	5.98	0.66

Table 6: Quantification of Rutin and Quercetin in the neem tea samples.

Sample code	Rutin (µg/mL)	Quercetin (µg/mL)
HC	432.9	4.6
NN	90.8	-
FG	1187.6	32.6
RG	634.2	12.8
JUL	617.6	13.4

Table 7: Microbial Growth on Agar Plate after Incubation.

Sample code	TABC	TFC	MSA	EMB	MAC	CA	SSA
RG	4.0×10^4	-	-	-	-	-	-
JUL	6.0×10^4	-	-	-	-	-	-
HC	7.0×10^4	-	-	-	-	-	-
NN	5.0×10^4	-	-	-	-	-	-
FG	TNTC	-	-	-	-	-	-

TABC: Total aerobic bacterial count; TFC: Total fungal count; MSA: Mannitol salt agar

EMB: Eosine methylene blue; MAC: MacConkey agar; CA: Cetrimide agar; SSA: Salmonella Shigella agar; TNTC: Too numerous to count; -: absent

6.7×10^6 cfu/g and fungal species ranging from 0.0×10^1 cfu/g to 7.1×10^6 cfu/g. The study also revealed the presence of some indicator pathogenic microorganisms which is in contrast with the present study. Summarily, it was observed that one sample or the other fell out of one or more specifications. There is need for regulatory agencies to put a bench mark that takes into account the total or cumulative number of tests passed or failed and ultimately surpasses individual tests. Campaigns should be encouraged on the consumption of well packaged herbal products.

CONCLUSION

The macro, microscopic, physicochemical, chemical and microbiological properties of neem products towards checks for identification and purity have been established. The neem products are generally considered as safe for consumption. These findings show the importance of post market surveillance and need for control procedures to determine which samples are eventually labelled as safe. The results obtained are beneficial towards enriching current monographs of the neem products for commercialization.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

WHO: World Health Organization; **HPLC:** High Performance Liquid Chromatography; **NIPRD:** National Institute for Pharmaceutical Research and Development; **TABC:** Total aerobic bacterial count; **TFC:** Total fungal count; **MSA:** Mannitol salt agar; **EMB:** Eosine methylene blue; **MAC:** MacConkey agar; **CA:** Cetrimide agar; **SSA:** Salmonella Shigella agar; **TNTC:** Too numerous to count.

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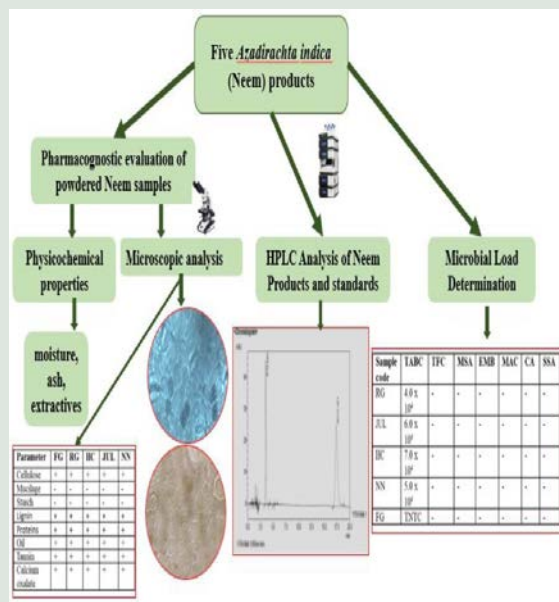
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GRAPHICAL ABSTRACT



SUMMARY

Market surveillance is essential to the proliferating use of herbal medicines. Good Agricultural, manufacturing, transportation and storage Practices must be encouraged and seen to be attainable. Finished herbal products must be well transported and stored in appropriate airtight packages. The absence of enteric pathogenic are central to consumption of quality herbal medicines.

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