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Phytochemical Composition, Antioxidant Activity, Stability, and Safety Evaluation of Herbal Tea Formulated from *Nephrolepis exaltata*

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ABSTRACT

Background: Herbal teas are increasingly valued for their therapeutic and nutraceutical properties due to bioactive phytochemicals with antioxidant and anti-inflammatory effects.

Method*:* Herbal tea produced from *Nephrolepis exaltata* was evaluated for its phytochemicals, macroscopic and pH assessment, proximate analysis, antioxidant capacity (DPPH radical scavenging, total antioxidant capacity, and ferric reducing antioxidant power), High-Performance Liquid Chromatography, and Fourier-Transform Infrared Spectroscopy using standard procedures. Accelerated stability studies were performed over 4 months at 40 °C/75 % RH. Acute oral toxicity of the herbal tea was assessed in female rats in accordance with OECD guideline 425. The hematological, biochemical, and histopathological parameters were assessed.

Result: Phytochemical analysis revealed the presence of flavonoids, phenolics, tannins, steroids, triterpenoids, and cardiac glycosides, while alkaloids, saponins, and anthraquinones were absent. Proximate composition indicated carbohydrates (44.6%), crude fiber (17.85%), lipids (14.5%), protein (3.94%), ash (5.3%), and moisture (13.5%). The tea exhibited mild acidity (pH 5.04–5.12) and strong antioxidant activity (DPPH inhibition of 86.5% at 250 µg/mL, TAC of 22.06µg/mL ascorbic acid equivalents, and FRAP of 94.27µg/mL). HPLC identified gallic acid (8.034mg/mL) and quercetin (8.834mg/mL) as dominant compounds, while luteolin and formononetin only appeared during storage. FTIR confirmed the presence of characteristic functional groups of polyphenols, lipids, and carbohydrates. Stability testing suggested preliminary stability under accelerated conditions. Acute toxicity studies demonstrated an LD_{50} >5000mg/kg, without significant alterations in body weight, organ morphology, hematological, or biochemical parameters.

Conclusion*:* The study characterised the phytochemicals of the herbal tea, evaluated its potency and safety, and established its preliminary stability under accelerated conditions.

Keywords**:** Acute toxicity, Antioxidant activity, Herbal tea, *Nephrolepis exaltata,* Phytochemicals.

1. INTRODUCTION

Since time immemorial, natural products have been the backbone of traditional healing systems worldwide and an integral part of history and culture [1]. Although the use of bioactive natural products as herbal drug preparations dates back hundreds, even thousands of years, their application as isolated and characterised compounds in modern

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drug discovery and development started only in the 19th century [2]. It has been well documented that natural products played critical roles in modern drug development. Among the many applications of natural products, herbal preparation remains an important means of delivering bioactive compounds for health promotion and disease management. One such preparation is herbal tea, which is widely consumed as a beverage and constitutes a common dietary practice in many African households [3]. Herbal teas are used as therapeutic vehicles in many forms of traditional medicine and are a popular global beverage [4]. Herbal teas are mixtures of several ingredients and are more accurately known as 'tisanes'. Tisanes are made from combinations of dried leaves, seeds, grasses, nuts, barks, fruits, flowers, or other botanical elements that give them their taste and provide the benefits of herbal teas [5]. Unlike most other forms of tea, herbal teas do not contain caffeine. They also taste great and are easy to drink. Most herbal teas may consist of a single main ingredient or a blend, intended to serve a specific purpose, such as relaxation, rejuvenation, or relief from a particular condition. It is also important to understand that there is a wide variety of herbal teas available on the market, each designed to provide a specific therapeutic or medicinal benefit. Herbal teas have long been valued for their therapeutic properties, cultural significance, and contribution to human health. Increasing scientific interest in plant-based infusions has highlighted their potential as sources of bioactive compounds, particularly phytochemicals with antioxidant and protective effects [6]. Among the diverse flora with medicinal promise, *Nephrolepis exaltata* (commonly known as the Boston fern) has attracted attention due to its rich phytochemical profile and traditional use in folk medicine [7]. Despite its widespread availability and ornamental appeal, systematic evaluation of its suitability as a functional herbal tea remains limited. Oxidative stress, driven by an imbalance between free radicals and antioxidant defenses, is implicated in the pathogenesis of numerous chronic diseases [8]. Plant-derived antioxidants, including phenolics, flavonoids, and tannins, play a crucial role in mitigating oxidative damage and promoting cellular health. Thus, exploring novel herbal tea formulations that harness these compounds is increasingly important. Beyond antioxidant potential, the stability and safety of such formulations are critical to ensuring consumer acceptance and long-term health benefits [6]. This study investigates the phytochemical composition, antioxidant activity, stability, and safety of a herbal tea formulated from *Nephrolepis exaltata.* By integrating chemical analysis, functional assays, and toxicity evaluation, the research aims to establish a scientific basis for its use as a safe, stable, and health-promoting beverage. These findings are expected to contribute to the expanding field of functional foods and support the development of innovative plant-based products for wellness and disease prevention.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Biological materials: Plant Collection, Identification and tea bag production

The leaves of *Nephrolepis exaltata* were collected from its natural habitat near the University of Lagos, Lagos State, Nigeria (6°30'48"N, 3°23'38"E) in March 2025. Identification and authentication of plant parts were carried out at the Herbarium of the Department of Botany, University of Lagos, Nigeria, by Dr. G.I. Nodza and voucher specimens were deposited, with the number LUH100220 assigned. The ingredients were dried in an oven at 45 °C until crisp and then pulverized. The samples were passed through a 2 mm mesh sieve, bagged using an automatic tea-bagging machine, and stored at room temperature (28 °C).

2.1.2 Chemicals and reagents

Distilled water, ferric chloride (Sigma Aldrich Germany), lead acetate, Dragendorff reagent (Merck), Wagner's reagent (Merck), Mayer's reagent (CDH Fine Chemical), Hager's reagent, Barfoed reagents, Fehling's A and B reagent (Sigma Aldrich Germany), 10% naphthol solution, hydrochloric acid, sulphuric acid (BDH Binder), acetic anhydride, 70%, 90% and absolute ethanol (EMD Milliform Corporation, Germany), 2,2Diphenyl-1-picrylhydrazyl (DPPH), chloroform (Merck), glacial acetic acid (GFS Chemicals, Inc., Columbus), standard α-Amylase, α-Glucosidase, Phosphate buffer, 1% soluble starch, maltose, A glucose estimation kit (GLUC-PAP) (RANDOX), dinitro salicylic acid, liver homogenate, acarbose, ascorbic acid, trichloroacetic acid, thiobarbituric acid (Sigma Aldrich Germany), ferrous sulphate (GHCL, India), sodium bicarbonate.

2.1.3 Equipment and other materials

UV spectrophotometer (Thermo Scientific BioMate 3, USA), automatic tea bagging machine (CECLE, China), thermometer (LAB100, Brannan, Wales), electrodes (Jenway, 3510, UK), FTIR spectrometer (Lumex (InfraLUM), Germany), Nikon Kohden centrifuge machine (DF 12, DragLab, China). Nikon light microscope, swelab auto

counter (Boule Diagnostics, Sweden), cages, bedding, rat feed, oral cannula, syringes, surgical knives, forceps, surgical blade, EDTA sample bottles, non-heparinised centrifuge sample bottles, biochemical assay kits, antioxidant assay kits and analytical balance.

2.2 Methods

2.2.1 Preparation of aqueous infusion of Herbal tea

Five hundred grams (500 g) of herbal tea (containing leaves of *N. exaltata:* 100 %) was brewed in 3 L of boiled water for 2-3 mins to extract the bioactive constituents, after which it was filtered [9]. The filtrate was collected, allowed to cool, and administered to the animals. The aqueous extract was used for phytochemical screening, antioxidant assay, and oral acute toxicity [6].

2.2.2 Phytochemical screening

Qualitative phytochemical screening tests were carried out on the herbal tea samples from *Nephrolepis exaltata* leaves to determine the presence of phenolic compounds, reducing sugars, steroids, saponins, tannins, alkaloids, flavonoids, cardiac glycosides, combined anthraquinones (modified Borntrager's test), and triterpenoids. The presence of bioactive constituents in the herbal tea was determined using standard methods [10].

2.2.3 pH in cold water, room temperature, and hot water

A tea bag was infused in 250 mL of water at different temperatures for about 30 minutes: cold (10 °C), room temperature (28 °C), and hot (50 °C), with the temperature measured with a thermometer (LAB100, Brannan, Wales). The pH readings were measured using the electrodes (Jenway, 3510, UK) immersed in the volumetric flask containing the infused tea. The readings were taken and recorded appropriately for the three categories [11].

2.2.4 Antioxidant assays

2.2.4.1 2,2-Diphenyl-1-picryl hydrazyl (DPPH) radical scavenging assay.

The antioxidant activity of the extract was evaluated on the basis of the radical scavenging effect of the stable 2,2 -diphenyl-1-picrylhydrazyl (DPPH) free radical activity, in comparison with the ascorbic acid standard, by a slightly modified method [12]. A stock solution of the extract was prepared by dissolving 100 mg of the aqueous extract in 100 mL of methanol. Varying concentrations of the extract, 10 mg/mL, 50 mg/mL, 100 mg/mL, 150 mg/mL, and 200 mg/mL, were prepared by carrying out serial dilutions. A solution of the radical was also prepared by dissolving 3.94 mg of DPPH in 100 mL of methanol. Methanol (3 mL) with DPPH solution (0.1 mM, 1mL) was used as a control. The same procedure as with the ascorbic acid standard was carried out with each of the extract samples [13]. Percentage (%) inhibition was calculated using Equation 1

$$Percentage\ inhibition = (Ac - Aa)/Ac * 100\% \quad \text{Equation 1}$$

Where: A_c is the absorbance of the control sample, and A_a is the absorbance of the extract sample.

For each sample, the concentration with 50% scavenging effect (EC_{50}) on the DPPH radical was determined by interpolation of linear regression analysis.

2.2.4.2 Total Antioxidant Capacity (Phosphomolybdic determination of Total Antioxidant Capacity)

The total antioxidant capacities of the herbal tea sample were evaluated by the Phosphomolybdenum method. The assay is based on the reduction of Mo (VI) to Mo (V) by the sample and subsequent formation of a green Phosphate/Mo (V) complex at acidic pH. Aliquots of 10, 25, 50, 100, and 250 µg/mL of standard ascorbic acid were prepared. The absorbance was measured at 695 nm using a UV spectrophotometer (Thermo Scientific BioMate 3, USA). Determinations were done in triplicate using a one-point assay with extrapolation from the calibration plot. Total antioxidant capacity of the herbal tea was extrapolated from the calibration curve of the standard [14].

2.2.4.3 Determination of Antioxidant Activity Using the Ferric Reducing Antioxidant Power (FRAP) Method.

The FRAP assay was conducted following the modification of the method described by Saka *et al*. [14]. Three milliliters of prepared FRAP reagent were mixed with 100 µL of diluted sample; the absorbance at 593 nm was

recorded after a 30 min incubation at 37 C. Aliquots of standard at concentrations of 0.2, 0.4, 0.6, 0.8, 1.0 mg/ml were prepared. Determinations were done in triplicate using a one-point assay with extrapolation from the calibration plot. The change in absorbance was measured at 593 nm using a UV spectrophotometer (Thermo Scientific BioMate 3, USA). This method measures the ability of antioxidants to reduce ferric iron. It is based on the reduction of the complex of ferric iron and 2,3,5-triphenyl-1,3,4-triaza-2-azoniacyclopenta-1,4- diene chloride (TPTZ) to the ferrous form at low pH [12].

2.2.5 Proximate Analysis

Proximate analysis of herbal tea samples, including total carbohydrates, crude lipid, crude protein content, moisture content, crude fiber content, and total ash content, was estimated using standard methods [15, 16]. The extractive values of the tea were also estimated [17].

2.2.6 Accelerated Stability Studies

The experimental setup used for this study comprised temperature/humidity combinations tested for these samples. Tea bags were stored in a stability chamber at 40 °C/75 % Relative Humidity (40 °C/75 %RH) for 4 months. Samples were withdrawn from the chamber at the end of each month for High-performance liquid chromatography (HPLC) fingerprints and analysis [18].

2.2.7 HPLC Analysis; Sample Preparation and Analysis

A 250 mg portion of the content of a tea bag was weighed into labeled plastic bottles and extracted with 5 mL of methanol. The mixtures were sealed and sonicated for 5 minutes. After filtration through a 0.45 µm Millipore filter, 2 mL of the filtrate was collected in separate vials, while a methanol-filled vial served as the blank control. The vials were placed in the HPLC vial rack, and each sample was analysed in duplicate with a 7-minute run time. The standards (gallic acid, quercetin, luteolin, and formononetin) were used to analyse the chromatogram at concentrations ranging from 10 to 100 µg. The chromatographic analysis was performed using a reverse-phase HPLC system equipped with an Agilent Eclipse XDB C18 column (150 × 4.6 mm, 5 µm particle size). The mobile phase consisted of methanol: acetonitrile: water (40:15:45, v/v/v) containing 1% (v/v) acetic acid, delivered at a flow rate of 1.0 mL/min under isocratic conditions. The column temperature was maintained at 25 °C, and the injection volume was 10 µL. Detection was carried out using a UV detector set at a wavelength of 257 nm. The validation for the HPLC method used to quantify the herbal tea were calculated (Linearity, Limit of detection (LOD), Limit of Quantification (LOQ) and Repeatability).

2.2.8 Fourier Transform Infrared (FTIR) spectroscopy

Fourier transform infrared (FTIR) spectrometry was used to analyse and characterise the functional groups present in the tea sample. A small amount of crude extract was placed on the germanium piece of the FTIR spectrometer (Lumex (InfraLUM), Germany), with the absorbance data collected over the wavenumber range from 500-4000 cm^{-1} [19].

2.2.9 Acute toxicity study

2.2.9.1 Procedures and experimental conditions

The toxicity tests were carried out according to the Organisation for Economic Co-operation and Development (OECD) test guideline, i.e., OECD Test Guideline 425, with slight modifications [20]. The rats were obtained from the Animal House Unit, Lagos University Teaching Hospital, University of Lagos. Non-pregnant and nulliparous rats, aged 8–12 weeks (150 ± 20g), were randomly selected and maintained under normal conditions for at least 7 days to adapt to laboratory conditions before the experiments and housed in groups of three for acute oral toxicity. Before the experiment, each animal's body weight was recorded to determine the appropriate treatment dose. The rats were given standard rat pellets and reverse osmosis (RO) water *ad libitum*. The rats were maintained at a room temperature of 28 °C, with a 12 h light/dark cycle.

2.2.9.2 Ethical Approval

All procedures used in the present study were conducted in accordance with the guidelines of the College of Medicine, University of Lagos Health Research Ethics Committee (CMULHREC). The research was carried out after approval from the CMUL/ACUREC (Ref: CMUL/ACUREC/08/25/2027). In accordance with OECD Test

Guideline 425 (Up and Down Procedure), nulliparous and non-pregnant female rats were randomly selected. Animals were kept under standard conditions for 7 days. A limit test was performed at 2000 mg/kg p.o. as a single dose, and rats were kept without food for 3–4 h before dosing but had access to water ad libitum. The dose was administered to a single female rat according to body weight. The animals were closely observed for the first 30 min, then for 4 h. Food was provided after 1–2 h of dosing. After the survival of the treated rat, four (4) additional rats were administered the same dose under the same conditions. The same procedure was followed for the vehicle-treated control group of 5 rats, to whom distilled water was administered in the same volume as that of the treated group. Both groups were observed closely for any toxic effects within the first 6 h and then at regular intervals for a total period of 14 days. Surviving rats were observed to determine the onset of toxic reactions. Weights of animals were monitored and documented as well. An acute toxicity test was also performed at a dose of 5000mg/kg body weight, and this is because there is a strong likelihood that the results of such a test could have direct relevance for protecting human or animal health and the environment. One rat is dosed 5000mg/kg of body weight and kept under observation. After the survival of the animal, two additional rats were dosed under the same conditions. The rats were kept under full 14-day observation without dosing of further animals. All animals were kept under the same natural conditions and observed for signs of toxicity and mortality for 72 hours, and for the next 14 days for any deaths or changes in general behavior and other physiological activities [21]. The volume to be administered to each animal was calculated using Equation 2.

$$Volume(ml) = \frac{\left[Weight\ of\ animals\ (kg) * Dose\left(\frac{mg}{kg}\right)\right]}{Concentrations\left(\frac{mg}{ml}\right)} \qquad Equation\ 2$$

All observations, including changes in skin and fur, eyes and mucous membranes, and behavioral patterns, were systematically recorded and maintained in an individual record. In addition, consideration was given to observations of convulsions, tremors, diarrhoea, salivation, lethargy, sleep, coma, and mortality. The body weights of the animals were recorded shortly before administration of the test substance and every 3 days thereafter. At the end of the experiment, all animals were humanely sacrificed, and the organs were isolated, weighed, and conserved in 10% buffered formalin for further study.

2.2.9.3 Haematology and Serum Biochemistry

At the end of each experiment (on the 15th day of the acute oral toxicity test), a blood sample (5 mL) was obtained by ocular puncture of the orbital sinus using a disposable capillary tube. Blood were collected by retro-orbital plexus using capillary tubes into K_2EDTA tube for analysing hematological parameters such as haemoglobin (HGB), white blood cell (WBC), Neutrophil, Lymphocyte, Monocyte, Eosinophil and Basophils and in Plain tubes for biochemical parameters such as Urea, Creatinine, Albumin, Globulin, Total bilirubin, Conjugate Bilirubin, Alkaline phosphatase (ALP), Alanine transaminase (ALT) and Aspartate aminotransferase (AST). The blood samples placed in plain tubes were left at room temperature (28 °C) for 15-20 min to promote coagulation. The blood samples were centrifuged at 5000 rpm for 20 min at 4 °C using a Nihon Kohden centrifuge (DF 12, DragLab, China). The serum obtained was then analysed using the improved Swelab auto counter by Boule Diagnostics, Sweden. Immediately after blood collection, rats were humanely sacrificed, and the liver, kidney, and heart were harvested [22].

2.2.9.4 Histopathological study

Necropsy was performed on the animals from the acute oral toxicity study on day 15. After the blood collection, rats were sacrificed, and the vital organs (liver, kidney, and heart) were extracted. The organs were cleaned of adherent fat and blotted dry. They were fixed in 10% buffered formalin, routinely processed, and embedded in paraffin wax. 5-µm-thick paraffin sections were cut on glass slides and stained with haematoxylin and eosin. An experienced pathologist, blinded to the treatment group, conducted the analysis. The slides were examined under a Nikon light microscope [23].

2.3 Statistical Analysis

Data were obtained in triplicate and expressed as Mean ± SEM. Statistical analysis of the results was performed using one- and two-way analyses of variance (ANOVAs), followed by Dunnett's post hoc test, as well as regression analysis, using GraphPad Prism 9.5.0 (Dotmatics, San Diego, CA, USA) (*=p<0.05, **=p<0.001).

3. RESULTS

3.1 Percentage yield and Phytochemical screening

The percentage yield (extractive value) of the extract was calculated to be 18.6%. The preliminary phytochemical analysis of the aqueous extract of herbal tea (Table 1) revealed the presence of phytoconstituents in the herbal tea, as indicated in the table.

Table 1: Phytochemical screening of the herbal tea sample

Test	Inference
Alkaloids	-
Tannins	+
Phenols	+
Flavonoids	+
Saponin	-
Cardiac Glycosides	+
Anthraquinones	-
Terpenoids	+
Triterpenoids	+
Steroids	+
Carbohydrates	+

NB: + means present; - means absent

3.2 PH analysis

The pH tests carried out on herbal tea in hot and cold water, which are used to determine the acidity and alkalinity of the tea sample at varying temperatures, are shown in Table 2 below.

Table 2: pH value of herbal tea in water at different temperatures

Sample	pH in Hot Water (50 °C)	pH in Water (28 °C)	pH in Cold Water (10 °C)
Herbal tea	5.05	5.04	5.12

3.3 Antioxidant assay

3.3.1 DPPH assay

The scavenging ability of herbal tea for DPPH was presented in Table 3. IC_{50} for sample is 58.06 with regression equation of $y = 0.3026x + 21.63$, $R^2 = 0.9834$ while for standard is 3.23 with regression equation of $y = 0.1947x + 59.14$, $R^2 = 0.9993$.

Table 3: Antioxidant effect of herbal tea sample and ascorbic acid (standard) using DPPH assay

Concentration (µg/mL)	Inhibition (%) ± SEM (Standard)	% inhibition ± SEM (herbal tea)
10	72.5 ±0.23	22.9 ±0.80
25	86.6 ±0.17	32.6 ±0.27
50	92.2 ±0.12	45.7 ±0.40
100	93.9 ±0.10	73.7 ±0.23
250	94.3 ±0.13	86.5 ±0.12

3.3.2 Total Antioxidant Capacity

Total Antioxidant Capacity of the extract was obtained as 22.06 µg/mL equivalents of ascorbic acid, obtained from the TAC calibration curve of ascorbic acid (Figure 1).

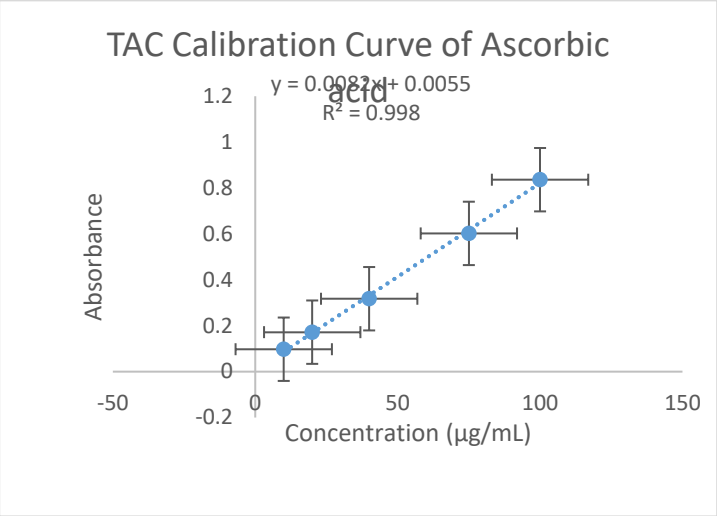

Figure 1: Calibration curve of Ascorbic acid standard for TAC assay

3.3.3 Ferric Reducing Antioxidant Power (FRAP) Assay

The ferric reducing antioxidant power (FRAP) of the herbal tea sample was determined to be 94.27 µg/mL, derived from the $FeSO_4$ calibration curve (Figure 2).

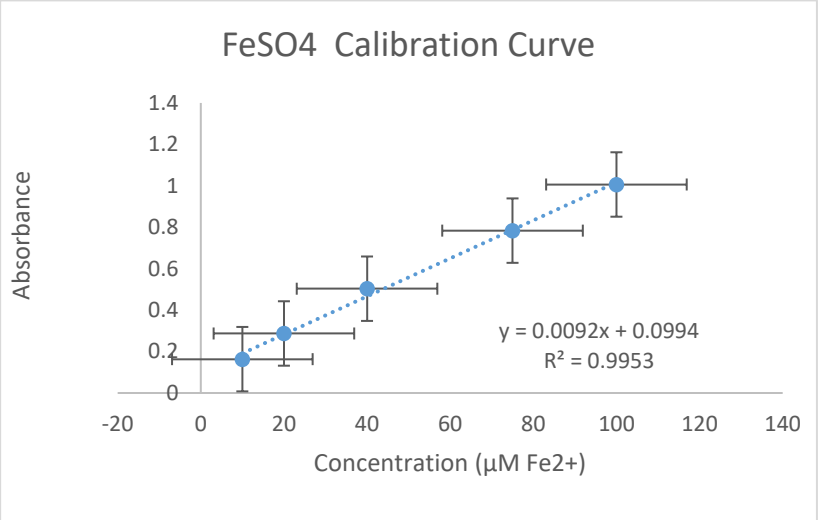

Figure 2: FRAP Calibration curve

3.4 Proximate analysis

Proximate analysis is used to assess the quantitative and qualitative composition of herbal teas to identify and estimate their nutritional value. The composition revealed carbohydrates (44.6–44.9 %), crude fiber (17.4–18.3 %), crude lipids (14.5–15.0 %), crude protein (3.94 %), ash (5.2–5.3 %), and moisture (13.5 %) (Table 4).

Table 4: Proximate Analysis of the herbal tea obtained from *Nephrolepis exaltata*

Parameter	Composition (%) ± SEM
Moisture content	13.5 ± 0.00
Total ash	5.3 ± 0.05
Crude fibre	17.9 ± 0.40

Crude protein	3.94 ± 0.00
Crude lipid	14.8 ± 0.25
Carbohydrate	44.7 ± 0.15
Water extractive value	18.6% ± 0.70
Alcohol extractive value	6.3 ± 0.30

3.5 HPLC assay

The validation summary for the HPLC method used to quantify the herbal tea are presented in table 5a while the chromatographic data for the standards, including peak areas (milli-absorbance units per second (mAU/s)) and concentrations (µg/mL), are summarised in Table 5b. These data serve as a basis for identifying and quantifying the corresponding compounds in the herbal tea samples presented in Table 6.

Table 5a: Validation summary for the HPLC method used to quantify the Herbal Tea

Compound	**Linearity R^2**	**LOD (µg/mL)**	**LOQ (µg/mL)**	**Repeatability %RSD**
Formononetin	0.996	8.556	25.927	-
Gallic Acid	0.995	8.725	26.439	1.74%
Quercetin	0.995	8.759	26.542	1.43%
Luteolin	0.996	8.603	26.069	-

Table 5b: HPLC analysis of Standard Compounds

	AREAS (mAU*s)	**AREAS (mAU*s)**	**AREAS (mAU*s)**	**AREAS (mAU*s)**
Concentration (µg/Ml)	Gallic Acid	Quercetin	Luteolin	Formonometin
10	130.8	297.5	280.3	191.3
20	240	567.1	532.6	364.2
40	448.6	1074.5	1014.9	700.2
60	714.2	1721.9	1631.5	1123.8
80	896.8	2160.4	2068.0	1424.7
100	1210.8	2924.5	2794.8	1926.7

Table 6: HPLC accelerated stability studies of the herbal tea for four months.

Parameters	**Calibration Equation**	**Before Incubation**	**1st month (µg/mL)**	**2nd month (µg/mL)**	**3rd month (µg/mL)**	**4th month (µg/mL)**
Gallic Acid	Y= 11.752x	8428.63	7941.91	14471.10	1532.67	ND
Quercetin	Y= 28.321x	4046.03	4869.34	60213.33	6621.76	15614.60
Luteolin	Y=18.61x	8050.00	ND	ND	1346.43	40883.28
Formonnetin	Y= 27.007x	ND	ND	684.06	931.23	ND

Note: ND: Not detected

3.6 Acute toxicity study

In the acute toxicity study, the results obtained were summarised in Tables 7 and 8. Table 7 represents the biochemical analysis of blood samples of the rats, while Table 8 represents the results of the haematological assay following the acute oral toxicity test of *Nephrolepis exaltata* in rats. Figure 3 shows the effect of herbal tea extract on the liver, kidney, and heart, showing mild vascular congestion with normocellular glomerular tuft (stained with Hematoxylin and eosin 100).

Table 7: Biochemical analysis of blood samples of rats

Biochemical Parameters	Control	2000 mg/kg	5000 mg/kg
AST (U/L)	253.67 ± 12.66	272.23 ± 25.11	268.87 ± 24.89
ALT (U/L)	95.60 ± 3.73	95.83 ± 5.61	95.27 ± 6.05
ALP (U/L)	217.70 ± 79.61	205.6 ± 33.27	135.97 ± 55.15
TP (g/L)	65.27 ± 8.74	74.03 ± 12.1	80.73 ± 5.16
ALB (g/L)	33.27 ± 7.02	37.6 ± 0.96	41.47 ± 3.38
T. BIL (µmol/L)	9.77 ± 0.93	10.93 ± 1.01	9.43 ± 0.89
D. BIL (µmol/L)	1.50 ± 0.17	1.5 ± 0.20	1.57 ± 0.25
UREA (mmol/L)	5.37 ± 0.47	6.27 ± 0.25	6.2 ± 1.44
CREAT µmol/L	93.67 ± 26.26	85.8 ± 37.76	112.4 ± 26.91
Na (mmol/l)	146.17 ± 1.36	145.4 ± 1.22	146.73 ± 1.4
K (mmol/l)	5.74 ± 0.24	5.45 ± 0.24	5.68 ± 0.17
Cl (mmol/l)	107.4 ± 3.65	106.97 ± 1.65	109.7 ± 0.56
HCO_3 (mmol/l)	12.23 ± 2.48	13.07 ± 0.83	13.03 ± 1.3
T. CHOL (mmol/L)	2.23 ± 0.31	2.5 ± 0.1	2.3 ± 0.62
TRIG (mmol/L)	0.7 ± 0.2	0.83 ± 0.45	0.97 ± 0.29
HDL (mmol/l)	1.17 ± 0.21	1.27 ± 0.12	1.2 ± 0.26
LDL (mmol/l)	0.77 ± 0.06	0.83 ± 0.15	0.63 ± 0.35

The values represent mean ± SEM (*=p<0.05, **=p<0.001)

Key: AST: Aspartate Aminotransferase, ALT: Alanine Aminotransferase, ALP: Alkaline Phosphatase, TP: Total Protein, ALB: Albumin, T. BIL: Total Bilirubin, D. BIL: Direct Bilirubin, UREA: Urea, CREAT: Creatinine, Na: Sodium, K: Potassium, Cl: Chloride, HCO3: Bicarbonate, T. CHOL: Total Cholesterol, TRIG: Triglycerides, HDL: High-Density Lipoprotein, LDL: Low-Density Lipoprotein.

Table 8: Haematological analysis assay for acute oral toxicity test of N*ephrolepis exaltata* in rats

Hematological Parameters	Control	2000 mg/kg	5000 mg/kg
WBC × 10^3/µL	5.4 ± 2.40	5.67 ± 1.94	6.37 ± 4.41
Lymphocyte %	51.2 ± 9.10	39.10 ± 9.77	41.67 ± 10.91
lymphocyte (%)	2.8 ± 0.30	2.10 ± 0.40	2.33 ± 0.98
RBC ×10^6/µL	4.03 ± 0.05	4.61 ± 0.07	4.11 ± 0.77
HGB (g/dl)	8.5 ± 2.04	11.87 ± 0.75	9.87 ± 3.19
HCT (%)	29.9 ± 3.00	40.33 ± 1.25	34.00 ± 9.38
PLT ×10^3/µL	258 ± 40.50	265.00 ± 45.51	310.33 ± 127.56
MCV (fL)	74.4 ± 4.45	87.53 ± 1.54	82.03 ± 8.16
MCH (pg)	21.0 ± 2.50	25.67 ± 1.30	23.60 ± 3.25
MCHC (g/dl)	28.4 ± 1.20	29.33 ± 1.00	28.70 ± 1.45
Mid#	0.5 ± 0.12	0.53 ± 0.21	0.63 ± 0.32
Mid%	9.0 ± 3.81	9.03 ± 0.81	10.70 ± 4.87
Gran%	39.8 ± 9.45	51.87 ± 9.53	47.63 ± 12.35
Gran#	2.1 ± 2.33	3.03 ± 1.33	3.40 ± 3.40

RDW-CV (fl)	16.7 ± 1.23	11.87 ± 1.02	14.50 ± 1.71
RDW-SD (%)	44.8 ± 2.15	41.13 ± 2.06	45.40 ± 3.65
MPV $\times 10^3/\mu L$	10.0 ± 1.10	9.50 ± 1.10	9.30 ± 1.20
PDW (%)	15.2 ± 0.31	15.27 ± 0.31	15.23 ± 0.31
PCT (%)	0.258 ± 0.06	0.25 ± 0.06	0.288 ± 0.078

The values represent mean ± SEM (*=p<0.05, **=p<0.001)

Key: WBC: White Blood Count, Lymphocyte % / lymphocyte, RBC: Red Blood Count, HGB: Hemoglobin, HCT: Hematocrit, PLT: Platelet Count, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Hemoglobin, MCHC: Mean Corpuscular Hemoglobin Concentration, RDW-CV: Red Blood Cell Distribution Width (Coefficient of Variation), RDW-SD: Red Blood Cell Distribution Width (Standard Deviation), MPV: Mean Platelet Volume, PDW: Platelet Distribution Width, PCT: Platelet.

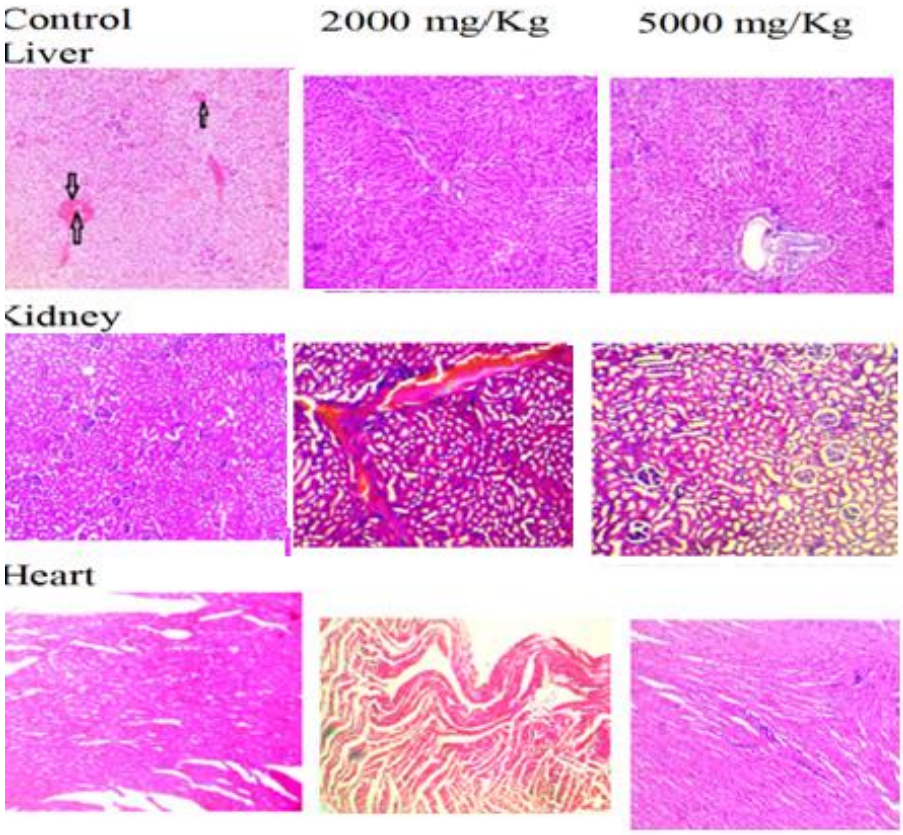

Figure 3: Effect of *N. exaltata* on the liver, kidney, and heart, showing mild vascular congestion with normocellular glomerular tuft (stained with Hematoxylin and eosin 100)

3.7 FTIR Assay

Figure 4 below depicts the decondensed and expanded FTIR spectra of the herbal tea, while Table 9 shows the FTIR spectrum readings for the herbal tea and its interpretation

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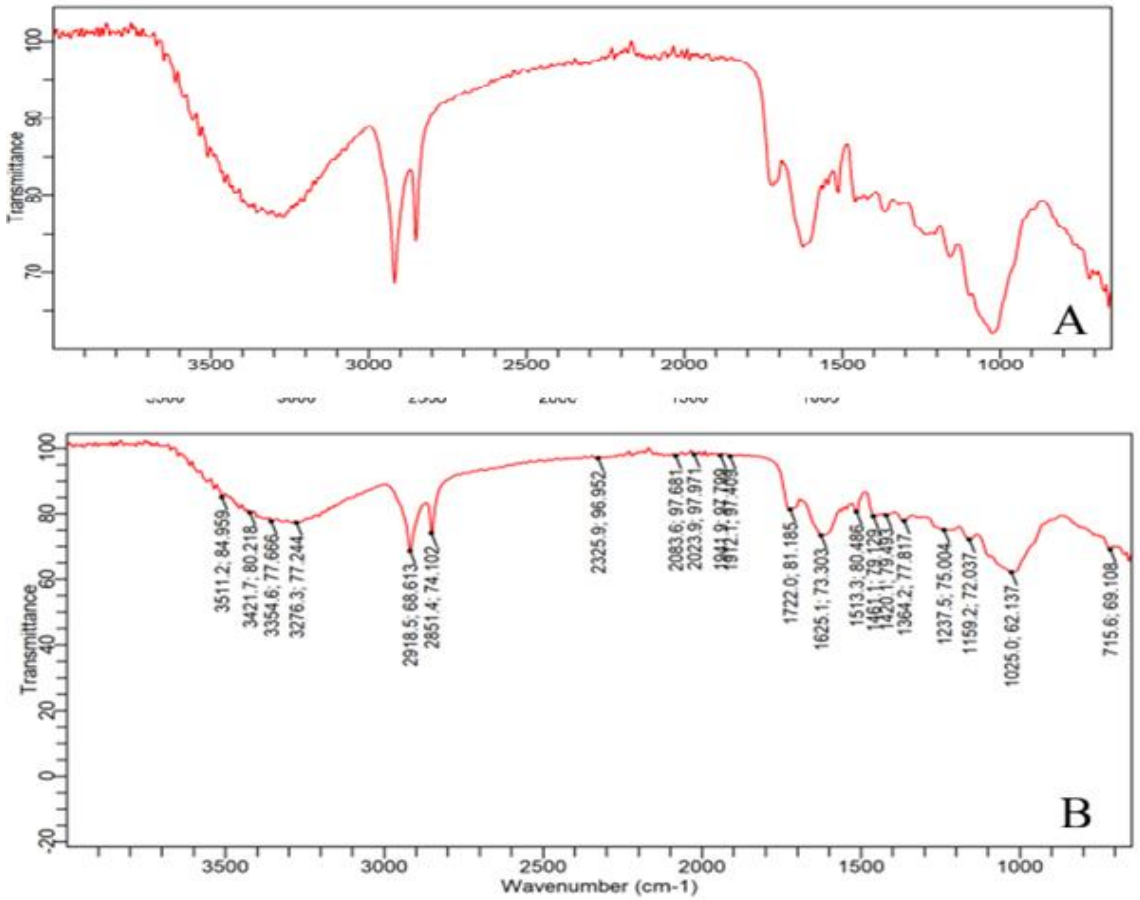

Figure 4: FTIR spectrum of herbal tea decondensed (A) and expanded (B) Note: Fig. 4B is the expanded spectrum of Fig. 4A showing the wavenumber.

Table 9: FTIR spectrum readings for herbal tea

Peak Number	Wavenumber(cm-1)	Intensity	Functional Group
1	715.64886	69.10776	C-H out-of-plane bending (aromatic rings or alkenes; common in caffeine or polyphenols).
2	1025.01791	62.13730	Strong C-O stretching (carbohydrates like cellulose; dominant in plant material, indicates fibres in tea leaves). This is the strongest absorption.
3	1159.20207	72.03739	C-O stretching (glycosides or alcohols in tea sugars/polyphenols).
4	1237.47616	75.00422	C-O stretching (ethers or phenolic compounds; linked to catechins).
5	1364.20565	77.81747	C-H or O-H bending (alkyl groups or phenols).
6	1420.11572	79.49281	C-H bending (CH_2/CH_3 in lipids or carbohydrate).
7	1461.11643	79.12910	C-H bending (CH_2/CH_3 in lipids or carbohydrate).
8	1513.29916	80.48601	C=C stretching (aromatic rings in caffeine or polyphenols).
9	1625.11930	73.30280	C=C stretching or amide I (aromatics/proteins; key for tea's flavour compounds).

10	1722.03008	81.18531	C=O stretching (carbonyls from esters or oxidized polyphenols; suggests some processing like in black tea).
11	1912.12431	97.40870	Random noise or a weak overtone.
12	1941.94301	97.79930	Weak alkyne or nitrile stretch (likely noise or minor impurity).
13	2023.94445	97.97059	Random noise or a weak overtone.
14	2083.58185	97.68059	Weak alkyne or nitrile stretch (likely noise or minor impurity).
15	2325.85881	96.95182	Weak alkyne or nitrile stretch (likely noise or minor impurity).
16	2851.41345	74.10197	Symmetric C-H stretching (aliphatic chains in lipids).
17	2918.50553	68.61339	Asymmetric C-H stretching (similar; stronger, from methyl/methylene groups).
18	3276.32996	77.24371	O-H stretching (part of broad band from hydrogen-bonded hydroxyls in polyphenols).
19	3354.60405	77.66604	O-H stretching (broad band continuation).
20	3421.69613	80.21771	O-H stretching (broad band; may include N-H from amino acids).
21	3511.15224	84.95875	O-H stretching (weaker edge of broad band; possibly free O-H).

4. DISCUSSION

The presence of flavonoids, tannins and phenolics in the tea sample containing *N. exaltata* can be relevant for their reported antioxidant and anti-inflammatory roles [24]; cardiac glycosides support potential cardioprotective claims, and the absence of anthraquinone suggests a reduced risk of laxative effects. These findings align with reports in the literature on polyphenols in teas and ferns [17], indicating that the formulation's spectrum of health benefits is enriched without toxic outliers. The pH evaluation is critical for ensuring the safety, stability, and bioavailability of herbal infusions, as extreme values can lead to enamel erosion or microbial proliferation. The tea exhibited mildly acidic pH values across all infusion temperatures. These findings suggest stability of the tea's acidity across a range of preparation conditions, with only slight elevation in cold infusion likely due to reduced solubility of acidic phytoconstituents at lower temperatures [25]. This mild acidity falls within the optimal range for palatability and safety, avoiding enamel erosion at pH values below 4.5 [6]. However, values in the microbial growth range (5.0–8.5) imply slight susceptibility to contamination during brewing, hence the need for hygienic practices. No outliers were observed, and the stability across temperatures supports the use of versatile preparation methods, enhancing consumer convenience and aligning with the literature on tea infusions [27]. The DPPH assay of the tea samples showed concentration-dependent radical scavenging, with inhibition ranging from 22.9 % at 10 µg/mL to 86.5 % at 250 µg/mL, comparable to ascorbic acid and attributed to phenolics [28]. TAC (22.06 µg/mL equivalents of ascorbic acid) and FRAP (94.27 µg/mL) indicate strong reducing power, validating claims linked to oxidative stress reduction [29]. The combined results from DPPH, TAC, and FRAP assays confirm the antioxidant potential of the tea. High carbohydrate content provides energy, while fiber supports digestive health, aligning with the literature on the gastrointestinal benefits of herbal teas [30, 31]. Moderate lipids enhance fat-soluble phytochemical absorption (e.g., terpenoids from *N. exaltata*) [32], and low protein is typical for infusions [33]. Ash indicates minerals for immune support [34] and moisture, though elevated, necessitates airtight packaging to prevent spoilage [16]. Implications include nutritional supplementation, but high fiber may affect bioavailability. Compared with review data (e.g., *N. exaltata* nutrient profile) [35], the tea demonstrates a balanced nutritional composition, implying therapeutic and nutraceutical synergy without toxicity risks. The extractive value of the herbal tea showed 18.3 % for the water extractive value and 6.6 % for the alcohol extractive value. This indicates good solubility of bioactive compounds in an aqueous medium, which is consistent with traditional tea infusion practices. The disparity between the water extractive index (18.3 %) and alcohol extractive value (6.6 %) highlights the

predominance of polar, water-soluble phytochemicals in the tea matrix. This is consistent with the traditional mode of tea consumption, where hot water serves as the extraction medium. The relatively lower alcohol extractive value suggests that while lipophilic compounds are present, they contribute less to the overall extractive profile. Such findings reinforce the nutritional and functional relevance of tea as a hydrophilic polyphenol-rich beverage, with implications for both consumer health and industrial processing. The analysis of the accelerated studies spanned four months (June to September 2025). HPLC profiling of herbal tea quantified gallic acid (8.403 mg/mL), quercetin (4.046 mg/mL), and luteolin (8.059 mg/mL). Chromatograms showed consistent retention times, but duplicate variability in the extraction effects. This broadens functional potential, aligning with the review of polyphenols' activities [33]. Accelerated studies (40°C/75% RH) revealed progressive degradation and observations suggest humidity- or temperature-induced changes, consistent with ICH Guidelines [36]. Based on results from accelerated stability studies conducted over four months, the herbal tea remained stable for two months, after which degradation began. Using Arrhenius extrapolation, the tea demonstrated preliminary stability under accelerated conditions [37], with a recommendation for airtight packaging and a minimum of 1-year expiry. The Fourier Transform Infrared (FTIR) spectroscopy analysis of the herbal tea samples provides a molecular fingerprint of the functional groups present in the formulation, derived from *N. exaltata* leaves. The spectra were compared with a previous study [38]. The peaks in the spectra correspond to functional groups present in the extracts. The spectra exhibited characteristic absorption bands indicative of bioactive phytochemicals, including polyphenols, flavonoids, terpenoids, and carbohydrates. These groups align with the tea's antioxidant and therapeutic properties, as confirmed by phytochemical screening and antioxidant assays in this study. In the Acute Toxicity Study, there was no mortality observed in the tested rats, and no physically observable signs of toxicity were detected during the 14-day experimental period, indicating that the herbal tea *i*s safe at the tested dose. Behavioral patterns of the rats during the acute toxicity test (fur and skin, eyes, itching, salivation, mucous membranes, urination (color), somatomotor activity & behavioral patterns, sleep, convulsions & tremors, respiration, coma, mortality) were all normal throughout the study. Although our data do not reveal clear dose-dependent weight variation effects between the 2000 and 5000 mg/kg groups, the absence of weight loss, even transiently, suggests that neither dose induced overt systemic toxicity. Body weight is a sensitive indicator of acute toxicity; rapid loss (>10% of baseline weight) typically signals systemic distress or organ dysfunction [35]. The stability observed here, across all dosage groups, implies preserved general well-being, metabolism, and sufficient feed intake during the study window. There was no statistically significant difference in biochemical parameters when compared with the control. The liver function markers (ALT, AST) were normal within the tea and vehicle (distilled water) groups. No significant differences in triglyceride, LDL, or cholesterol were observed among groups. No significant difference in HDL levels was found between the herbal tea-treated rats and control animals. Histological evaluation of liver, kidney, and heart tissues following acute administration of herbal tea revealed predominantly normal architecture, with only limited deviations. In the control group, the liver sections demonstrated vascular congestion with periportal edema, while both kidney and heart tissues appeared unremarkable, showing intact glomerular tufts, renal tubules, and myocardial fibers. At the 2000 mg/kg dose, the liver and heart were normal, but the kidney showed vascular congestion without tubular necrosis or glomerular injury. At the highest tested dose of 5000 mg/kg, the liver, kidney, and heart were histologically normal, with radially arranged hepatocytes, normocellular glomeruli, viable renal tubules, and preserved cardiac myocytes. The heart remained structurally normal in all groups, with no evidence of myocardial degeneration, inflammatory infiltration, or vascular abnormalities. This consistency across groups indicates that the herbal tea does not exert acute cardiotoxicity, even at high doses. Comparable outcomes have been reported in studies of other medicinal plants, where cardiac tissue is typically preserved in acute settings unless the compounds directly interfere with contractility or cellular metabolism [39]. Acute administration of *N. exaltata* up to 5000 mg/kg did not produce structural injury in the liver, kidney, or heart. The incidental vascular congestion noted in the control liver and the 2000 mg/kg kidney appears not to be treatment-related, given the absence of lesions at the higher dose and the maintenance of normal architecture in other organs [40]. These results, when taken together with the stability of body weight trajectories, suggest that the tea is well tolerated in acute exposure and does not exert overt organ toxicity at the tested doses. There was no statistically significant difference in biochemical and hematological parameters compared with the control, suggesting that the herbal tea did not significantly affect the rats' profiles of these parameters. Across all groups, including the control group, rats exhibited normal weight trajectories during this period. No abrupt declines or abnormal fluctuations exceeded typical biological variation, suggesting the tea did not provoke overt toxicity. The biochemical assay results for this acute toxicity study of the herbal tea indicate generally preserved liver and kidney function, with only modest

fluctuations in some markers and no consistent dose-dependent deterioration or structural damage, despite biochemical variation in the histology slides. Specifically, aminotransferases (AST, ALT) and ALP show moderate levels across control, 2000 mg/kg, and 5000 mg/kg groups; bilirubin (total and direct) remains stable; total protein and albumin are slightly elevated in treated groups in some individuals; creatinine and urea values show variation, especially in creatinine at higher doses, but do not uniformly exceed expected ranges; electrolytes are largely normal; lipid profile markers (cholesterol, triglycerides, LDL, HDL) sit within expected physiological limits without consistent increase or decrease. These outcomes suggest that at both tested doses, the tea does not induce acute hepatic or renal damage severe enough to disrupt biomarker homeostasis. The stability of bilirubin suggests that cholestasis or severe hepatic dysfunction is unlikely. Minor elevations in ALP and occasional fluctuations in creatinine may reflect mild, transient changes or adaptive responses rather than pathology. The hematological findings of the present study indicate that the herbal tea does not cause consistent, dose-dependent alterations in blood parameters under acute conditions. Occasional outliers were noted, particularly at 5000 mg/kg, but these did not correspond with growth suppression or structural organ injury. Within the broader toxicological framework, these results reinforce the herbal tea's apparent safety in acute exposure, while also underscoring the need for sub-acute and chronic evaluations to determine whether subtle hematological changes might emerge with repeated dosing.

5. CONCLUSION

This study validated the herbal tea's antioxidant efficacy, phytochemical richness, and stability. The tea demonstrated antioxidant potential, acceptable acute safety, and preliminary stability under accelerated conditions. FTIR confirms key functional groups associated with antioxidants. These results support the tea's potential to relieve oxidative stress. The phytochemical richness and antioxidant potential of *Nephrolepis exaltata* tea suggest its suitability as a functional beverage. Future work could focus on scaling production, optimizing formulations, and integrating it into nutraceutical markets.

DECLARATIONS

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Conflict of interests

The authors declare no conflict of interest.

Contribution of the Authors

AOO: Research concept and design, collection and/or assembly of data, writing the article, critical revision of the article; BOO: collection and/or assembly of data; data analysis and interpretation, final approval of the article; JOO: data analysis and interpretation, critical revision of the article; IJA: collection and/or assembly of data; IUP: collection and/or assembly of data; BAO: collection and/or assembly of data; OAS: collection and/or assembly of data.

AI Disclosure statement

No generative AI or large language model was used in the preparation of this manuscript.

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