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Biochemical and functional groups characteristics of tea blends from *Ficus capensis* and *Justicia secunda*

Nneka Nkechi Uchegbu[1], Temitope Omolayo Fasuan[2*], Nchekwube Love Onuoha[3] and Adefisola Bola Adepeju[4]

Abstract

Tea is one of the most consumed drinks in the world, second only to water, and is renowned for its wide range of health benefits.This study examined the development of tea blends from blends of *Ficus capensis,* and *Justicias ecunda* leaves. Dried leaves of *F. capensis and J. Secunda* were milled, sieved, and processed into tea using a three-level factorial design. The process was modelled and optimized. The tea was evaluated for physicochemical, phenolic compounds profile, antioxidants, and functional groups using standard procedures. The blood-boosting potentials of the herbal tea blends were investigated through animal experiment and haematological evaluations were carried out on the fed animals. The results gave the optimal extracts blends of 48 g/100 g of *F. capensis* and 52 g/100 g of *J. secunda.* Antioxidants showed 2,2-diphenyl-1-picryl-hydrazl value of 6464.95 ± 1.97 µmol Trolox equivalents/100 g, ferric reducing power (40.13 ± 0.07 mmol Fe^{2+}/100 g), and metal chelating (57.40 ± 0.62%). Physicochemical property showed total dissolved solids value of 6.51 ± 0.11 mg/g, *L (78.35 ± 0.42), *a (3.14 ± 0.09), and*b (13.57 ± 0.12). Phenolic compounds profile showed the presence of epicatechin (115.63 ± 0.052 mg/100 g), which have been associated with ability to improve healthy-living and lower the risk of some degenerative health issues. The *in vivo* results showed that developed tea was able to recover up to 95.28% of the packed cell volume, red blood cell (94.78%), and haemoglobin (97.52%), which compared well with the commercial product. The study showed the potential application of *F. Capensis* and *J. Secunda* leaves extracts in the formulation of functional beverage. The formulation procedures is scalable and has domestic and industrial applications. The tea blend could serve as a functional food ingredient and beverage to improve human health.

Keywords *Ficus capensis*, *Justicia secunda*, Factorial design, Optimization, Tea blends, Hematological indices, Functional groups

*Correspondence:
Temitope Omolayo Fasuan
temitopeomolayo@yahoo.com
Full list of author information is available at the end of the article

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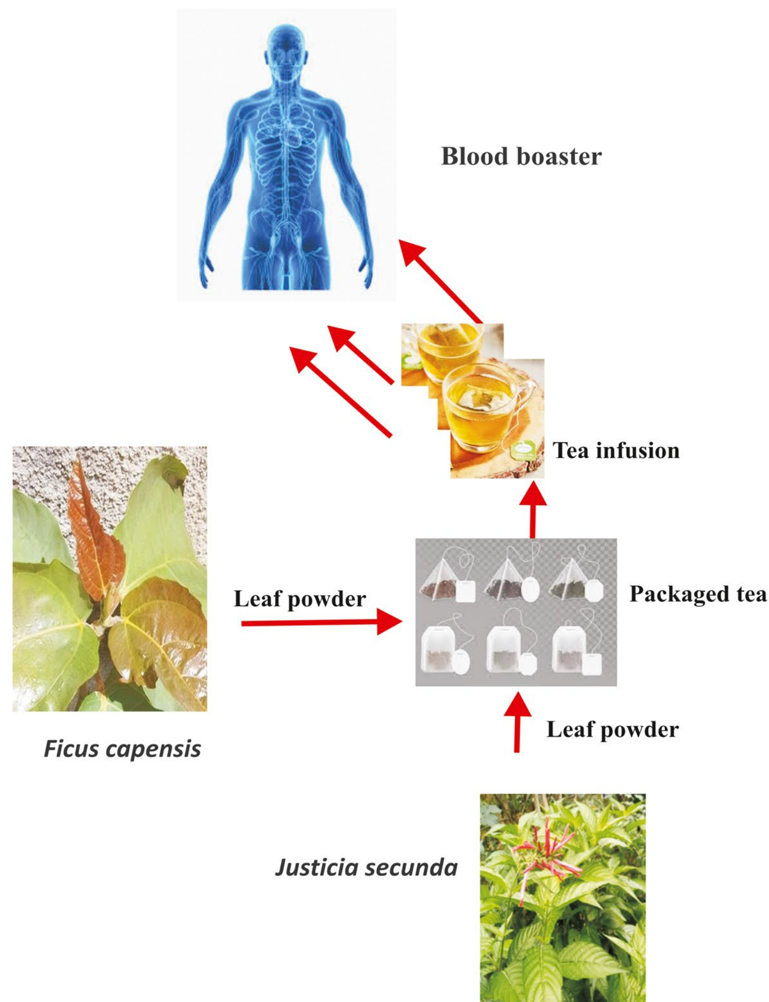
Graphical Abstract



Introduction

One of the most popular caffeinated and alcohol-free beverages is tea, which has a large global market. Tea is one of the most consumed drinks in the world, second only to water, and is renowned for its wide range of health benefits in addition to its pleasing sensory experience and pleasant flavor. Commercial tea products may be broadly divided into three main types based on the various production and fermenting processes (Meng et al. 2019). After the tea leaf is harvested, non-fermented tea, such as green tea, is often heated immediately to inactivate the polyphenol oxidase enzyme present in the leaves as well as the microflora. White tea, oolong tea, and black tea are a few examples of pre-fermented teas that have undergone varying degrees of polyphenol oxidase enzyme fermentation. White tea is a mildly fermented tea that just requires two basic processes: withering and drying.The withered leaves for black tea and oolong tea are rolled and crushed, which breaks cellular compartmentation and allows phenolic chemicals into touch with polyphenol oxidases to produce oligomers (Kongpichitchoke et al. 2016). Puer tea (ripe) and dark tea are examples of post-fermented tea, which are typically made from sun-dried green tea by microbial post-fermentation at higher temperature (about 50 °C) and humidity conditions, producing a special sensory quality through a series of oxidation, condensation, and degradation of tea polyphenols (Meng et al. 2019). Due to the presence of several kinds of chemical substances, such as polyphenols, alkaloids, proteins,minerals, vitamins, and amino acids, green tea has a very complex chemical makeup. The polyphenols d-catechin (C), (-)-epicatechin (E), (-)-epicatechingallate

(ECG), (-)-epigallocatechin (EGC), and (-)-epigallocatechin gallate (EGCG) are the primary active ingredients. Additionally, it has strong anticancer, antioxidant, anti-hypercholesterolemic, and antibacterial properties and is well regarded for its favorable effects on hematological issues. Commercial tea goods are typically produced from the young, tender leaves and buds of two commonly cultivated Camellia species (Theaceae), *Camellia sinensis* (L.) Kuntze and *Camellia sinensis* var. assamica (Masters) Kitamura (Meng et al. 2019). However, other leaves, such as *Ficus capensis*, *Justicia secunda*, can also be used to produce tea due to their medicinal properties. In Nigeria, each of *F. capensis* and *J. secunda* is traditionally being used as ablood booster. However, the science and composition of these two plants are yet to be documented.

Justicia secunda belongs to the family of Acanthaceae. In Nigeria, *J. secunda* is known as "Asindiri", which means medication that provides Blood (Osioma & Hamilton-Amachree 2017). *J. secunda* is employed to treat anemia. The *F. Capensis* leaf is called blood leaf, hospital too far, and blood tonic. The anemic patients in Africa typically take *F. Capensis* aqueous extract as tea (Ogunbamowo et al. 2020).

Ficus capensis, sometimes known as the fig tree, is a medicina plant that is widespread along Africa's coast and is particularly prevalent in Nigeria's forested regions. However, it is found largely in terrestrial zones along rivers. It has been viewed as an under appreciated plant and is known locally in Nigeria as Uwaryara, Opoto, or Akokoro. *F. capensis* has been used as a wound dressing, anemia therapy, and for dysentery in Nigeria. It is further used as an emollient for circumcision, leprosy, epilepsy, rickets, infertility, gonorrhea, oedema, and respiratory problems.Studies on phytochemistry have shown that this plant's leaf extract contains tannins, phytates, saponins, alkaloids, terpenoids, and flavonoids (Njoku-Oji et al. 2016). In addition to its traditional usage, *F. capensis* has been shown to have additional advantages based on current scientific research, including anti-sickling, antibacterial, antiabortifacient, immune-stimulatory, antidiarrheal, antioxidant, and pro-fertility in the treatment of azoospermia (Njoku-Oji et al. 2016).

Antioxidants are substances that prevent or slow the oxidation of other molecules by controlling the start or spread of oxidative chain reactions. Aantioxidants are free radical scavengers that shield the body from pathological conditions like anemia, asthma, arthritis, inflammation, neurodegeneration, aging, and possibly dementia. Free radicals can also cause anemia, asthma, arthritis, and other inflammatory conditions. It is becoming more widely accepted that diet may be a vital component of the defense against oxidative stress and damage by free radicals.As a result, some nutrients and dietary components with antioxidant qualities are crucial for the body's defense against oxidative stress damage. Recent consumer demands for natural foods with antioxidant properties that improve health and food preservation have led to a significant increase in the demand for antioxidants generated from natural resources (Neha et al. 2019; Lourenco et al. 2019; Gulcin 2020).

A collection of atoms known as a functional group is attached to the carbon skeleton of organic compounds and contributes certain features. Functional groups play an essential role in forming biological molecules, including proteins, carbohydrates, fats and oils, amino acids, and nucleic acids, among others. Each of these macromolecules has its specific functional groups, which affect how it behaves chemically and how it performs in living things. For example, methyl, hydroxyl, carbonyl, carboxyl, amino, phosphate, and sulfhydryl groups are some of the important functional groups in biological molecules; they have been shown to play crucial roles in the production of proteins, carbohydrates, lipids, and DNA molecules (Fasuan et al. 2021).

This study's goal was to look at the physicochemical, antioxidant, and functional group properties of blood booster tea blends from *J. secunda* and *F. capensis*.

Materials and method

Sources of rawmaterial

Fresh young leaves of the "*plants' F. capensis and J. secunda*" (Fig. 1) were collected from a certified farm in the Nigerian town of Nsukka in May 2022. They were then brought to the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, for verification.

Preparation of *Ficus capensis* and *Justicia secunda* leaves

F. capensis and J. secunda's freshly picked leaves (2 kg each) were carefully separated and washed with distilled water (1:100, w/v) to eliminate dirt and stick elements. The samples were manually cut into 5 mm pieces using a table knife and meter rule after being allowed to drain. The leaf pieces were steam and dried at 50 °C for 6 h in a hot air oven (SM9053, China). Using an attrition mill and an aluminum sieve, the dry samples were ground to a 2 mm aperture size. For future usage, the dried samples of *F. capensis* and *J. secunda* leaves were stored in an airtight container under refrigeration (4 °C) (Zhang et al. 2021; Zhao et al. 2013).

Experimental design

This study employed the three-level factorial design (3LFD) of the Response Surface Methodology (RSM). The amounts of *F. capensis* and *J. secunda* leaves were varied as independent variables (-1, 0, and + 1, where -1

Fig. 1 **a** *Ficuscapensis* (**b**) *Justiciasecunda*

Source: Esievo et al. (2018)

was the low level, 0 was the midpoint, and + 1 was the high level) (Table 1). At the same time, iron, total flavonoids, alkaloids, vitamin C, pyridoxine, taste, flavour, colour, aftertaste, and general acceptance were used as dependent variables (Table 1 and Table 2). The random experimental sequence was used throughout the experiment to avoid systematic errors. The coefficient of determination (R^2), adjusted coefficient of determination (Adj. R^2), probability value at 95% confidence interval, adequate precision, coefficient of variation, lack-of-fit, and analysis of variance (ANOVA) were used as statistical indices in the model fitting and evaluation process.

Table 1 Antioxidant, packed bulk density, and total dissolved solids characteristics of blood booster tea blends from *F. capensis* and *J. secunda*

Ficus capensis (g)	Justicia secunda (g)	DPPH (molTEAC/100 g)	FRAP (mmol Fe2 + /100 g)	Total Phenolic (mgGAE/100 g)	Packed bulk density (g/ml)	Total dissolved solids (mg/g)
1 (-1)	1 (-1)	2891.00 ± 1.73	16.80 ± 0.05	1168.37 ± 0.98	0.332 ± 0.02	2.85
2 (0)	1 (-1)	5607.00 ± 0.98	26.23 ± 0.10	1324.60 ± 0.35	0.336 ± 0.08	4.27
3 (+ 1)	1 (-1)	6480.00 ± 1.24	28.97 ± 0.32	1215.03 ± 1.05	0.340 ± 0.09	4.53
1 (-1)	2 (0)	2400.00 ± 1.04	17.23 ± 0.04	1204.60 ± 0.84	0.414 ± 0.07	2.70
2 (0)	2 (0)	5230.00 ± 1.06	33.41 ± 0.08	2203.83 ± 0.99	0.616 ± 0.10	4.80
3 (+ 1)	2 (0)	6480.00 ± 1.11	42.90 ± 0.70	2937.26 ± 0.93	0.694 ± 0.04	5.83
1 (-1)	3 (+ 1)	2200.00 ± 1.10	9.97 ± 0.02	860.03 ± 1.05	0.235 ± 0.02	1.97
2	3 (+ 1)	6170.00 ± 1.74	32.90 ± 0.82	2702.26 ± 0.86	0.570 ± 0.03	4.82
3 (+ 1)	3 (+ 1)	7420.00 ± 1.32	49.13 ± 0.09	4278.70 ± 1.32	0.782 ± 0.05	6.62
2 (0)	2 (0)	5230.00 ± 0.78	33.40 ± 0.17	2203.83 ± 1.70	0.588 ± 0.03	5.07
2 (0)	2 (0)	5230.00 ± 0.94	33.41 ± 0.23	2203.00 ± 1.25	0.590 ± 0.11	5.06
2 (0)	2 (0)	6231.00 ± 1.13	33.41 ± 0.45	2203.83 ± 0.68	0.589 ± 0.04	5.07
2 (0)	2 (0)	5230.00 ± 1.85	33.41 ± 0.10	2203.83 ± 0.19	0.588 ± 0.06	5.06

Values are means ± s.d ($n = 5$)

DPPH 2, 2-diphenyl-1-picrylhydrazyl, *FRAP* Ferric reducing powers, *TEAC* Trolox equivalent antioxidant capacity, *GAE* Gallic acid equivalent

Table 2 Model parameters of blood booster tea blends from *F. capensis* and *J.secunda*

Model parameters					
Model Terms	**DPPH**	**FRAP**	**Total Phenolic Content**	**Packed bulk density**	**Total dissolved solids**
Model	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
Lack-of-fit	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$
R^2	0.9721	0.9880	0.9167	0.9940	0.9991
Adj R^2	0.9550	0.9782	0.8340	0.9897	0.9985
CV (%)	0.92	1.16	1.52	3.25	0.14
Ad. Prec	19.89	87.39	20.34	48.32	90.29
Std. Dev	0.05	0.11	0.13	0.02	0.07

R^2 Coefficient of determination, *Adj. R^2* Adjusted coefficient of determination, *Adeq. Prec.* Adequate precision, *CV* Coefficient of variation, *Std. Dev.* Standard deviation, *DPPH—2* 2-diphenyl-1-picrylhydrazyl, *FRAP* Ferric reducing powers

Formulation and production of tea blends

As stated in Table 1, tea blends was developed using the leaves of *F. capensis* and *J. secunda*. To create a homogeneous mixture, each combination was sieved using a 2 mm mesh sieve. The prepared tea blends was first put into tea bags and a carton for a second packaging (Zhang et al. 2021; Zhao et al. 2013). Analysis of the created tea blends samples was done, and Design-Expert software version 10.0's multiple regressions were used to optimize the formulation process (USA).

Determination of loose tapped bulk densities and porosity

According to a standard approach, the loose and tapped bulk densities were collected in triplicate (World Health Organization 2012). Through a funnel, powder samples (weighing 50–70 g) were placed into a graduated glass cylinder (diameter 2.2 cm, capacity 250 cm^3). In every test, the powder filled the cylinder to a capacity of more than 60%. Next, the vessel was put into a tapping apparatus that enabled it to be repeatedly raised to a height of 3 cm and then let go (de Campos and Ferreira 2013). The powder volume was recorded regularly from $n = 0$ to $n = 1250$ taps, after which no more change in the powder volume was noticed. The assays were conducted at ambient temperature (28 ± 0.05 °C) with the relative humidity adjusted between 45 and 70%, which was enough to remove static charges altogether. By dividing the powder quantity in the cylinder by its volume, the loose (initial) and tapped (final) bulk densities was calculated. The void in the sample was calculated as porosity and expressed in percentage.

Determination of pH

To determine the pH of the tea, 10 g of the sample was dispersed in boiling 100 mL of distilled water, homogenized, allowed to boil for two minutes, cooled to ambient temperature (28 ± 0.05 °C) and allowed to stay for 30 min. Thereafter, it was filtered using mushlin cloth. The pH meter, which had previously been calibrated with buffer solutions of pH 4.0 and 7.0, was then used to determine the pH of the supernatant (Pekal and Pyrzbska 2015).

Determination of total dissolved solids

Using distilled water as the calibration medium, a hand refractometer (CareLab, India) was used to measure the total dissolved solids (TDS). To measure the TDS in °Brix in accordance with AOAC (2010) guidelines, a few drops of the solution tea and water (in a ratio of 1:1) were applied to the testing surface.

Determination of colour

With the use of a Minolta colorimeter (CR-200, Minolta Company, Ramsey, NJ, USA), the colour of tea sample (tea infusion) was measured (Drakos et al. 2017). The values for the CIELAB system's parameters for lightness L* (L = 100 means white; L* = 0 means black), chroma a* (+ a* means redness; -a* means greenness), and hue b (+ b* means yellow; -b* means blue) were calculated as the mean of three measurements made of each tea sample at three different locations.

Determination of DPPH radical scavenging activity

The antioxidant activity of our extracts was determined using the free radical DPPH as described by Naheed et al. (2017). To 160 L of DPPH produced in methanol, 40 L of various dilutions of the extracts (PE, DCM, EtOAc, and n-BuOH) or standard (butylated hydroxytoluene, BHT) were added. The resulting mixture was incubated in the dark for 30 min. The absorbance was then determined using a UV–VIS spectrophotometer at 517 nm, and the value of the DPPH was estimated.

Determination of ferric reducing antioxidant property

The ferric reducing capabilities of the samples were determined using a modified Álvarez et al. (2016)

technique. The FRAP working reagent was made by combining 300 mmol/L acetate buffer with a pH of 3.6, 10 mmol/L 2,4,6-tri-(2-pyridyl)-1,3,5-triazine, and 20 mmol/L $FeCl_3$ in the following ratios: 5: 1: 1, and heating the mixture to 37 ^{O}C. Distilled water was used to dilute the samples to 10 mg/mL. Then,40 ml of samples and 200 ml of FRAP reagent were put into a transparent, clean test tube, and absorbance was measured at 593 nm. As a control, iron II sulfate heptahydrate was utilized. From 1 mM of iron II sulphate, successive dilutions of 0.025 to 0.25 mM were made.The iron-reducing activity of the samples was evaluated using the Iron II sulphate heptahydrate standard curve, and the findings were reported in Fe^{2+} mMol.

Determination of metal chelating activities

The metal (iron) chelating activity (MCA) of the samples was measured using a modified Sellal et al. (2019) technique. In distilled water, the sample was produced for final concentrations ranging from 1.0 mg/ml to 5 mg/ml. After 1 h of hydration, the mixture was centrifuged (Bosh, TLD-500, England) at 3,000 g for 30 min at ambient temperature (28 ± 0.5 °C). In a reaction tube, a 1 ml aliquot of the sample solution or blank (distilled water) was combined with 50 ml of 2 mM$FeCl_2$ and 1.85 ml of double distilled water. Following that, 100 ml of 5 mM ferrozine was added. The mixture was completely vortexed and incubated at ambient temperature (28 ± 0.5 °C) for 10 min before measuring the absorbance values of the blank (Ab), and samples (As) at 562 nm with a spectrophotometer and the value of the metal chelating activity was then estimated.

Identification and quantification of phenolic compounds

Extraction was accomplished by suspending 0.6 g of material in 95% ethanol for 2 min, vigorously mixing for 2 min with a vortex mixer, and centrifuging for 30 min at ambient temperature (28 ± 0.5 °C) at 3,665 × g (0.502–1 Hospibrand, USA). Then, the residue was removed, leaving only the supernatant for identification and quantification. High-performance liquid chromatography was used to analyze the samples (Shimadzu Scientific, Japan). A Diode Array Detector (DAD) was used to scan the phenolic compounds in the sample at absorbance wavelengths ranging from 200 to 400 nm. Two solvent gradients (solvent A-water-acetic acid (96:4, v/v) and solvent B-acetonitrile) were utilized. The column's temperature was set at 30 °C, with a flow rate of 0.8 ml/min. The mobile phase contains standard phenolic solutions, and distinct calibration curves were used for phenolic compounds at five different concentrations.

Identification of functional groups

Using Fourier transform infrared (FTIR), the functional groups present in the tea were identified (Nicolet 470; Perkin Elmer Inc. Waltham, MA). The fine sample powder and potassium bromide (KBr) were combined to create the sample pellets (Sigma-Aldrich, FTIR grade). From 4,000 to 500 cm^{-1}, the spectra were captured in transmission mode. FTIR spectroscopy yielded an interferogram between wave number and transmittance.

Evaluation of blood boosting potential of the herbal tea

The blood-boosting potentials of the herbal tea blends were investigated through Wistar strain rats. The protocols for the study were reviewed and approved by the University of Nigeria (UNN/2016/237041), Nsukka, Nigeria.The study used adult male Wistar strain rats weighing 80 to 120 g. They were procured from the animal holding facility of the Department of Zoology and Environment Biology, University of Nigeria, Nsukka, Nigeria. Standard housing conditions (25 ± 2 ^{0}C and a 12-h light/dark cycle) were used for the animals. The rats were given regular pellets to eat once a day by Grand Cereals Ltd. in Enugu, Nigeria, and they had access to safe drinking water without restriction.

Design of anti-anemia experiments (in vivo assay)

Three sets of six male albino rats each were created from a total of eighteen male rats, receiving different dosed of samples and were labeled A, B, and C.Group A was induced with Cyclophosphamide injection and fed with the formulated blood booster tea. Group B was induced with Cyclophosphamide injection and fed with commercial blood tonic (control), while Group C was induced and fed with placebo.

Experimentally developed anemia was created in the rat by intraperitoneal injection for three days, during which blood was collected through Ocular puncture for an initial Hematological assay. The induced animals were fed with already prepared formulated feed (tea was mixed with animal feed at a ratio of 1:1 (tea:feed)). The feed was administered to the Group A animalsfor 28 days. Also, blood tonic (commercial product, which served as control) was administered orally to the Group B animalsonce daily for 28 days together with feed. After that, the blood was collected by Ocular puncture for hematological assay.

After the 28 days, the animals were sacrificed, and the region around the penile shaft was opened, the bladder was located, and the prostate around it was removed. The tissue (in normal saline) was fixed using 10% formalin. The tissues were analyzed histologically using a microtome after 24 h, followed by hematoxylin and eosin

staining. Under a microscope, the slides were examined, and the images were captured.

Furthermore, red blood cells (RBC) count was carried out as described by Prudent et al. (2015). A 3.98 L of 10% sodium citrate were thoroughly mixed with 20 µl of whole blood. By holding the pipette vertically, the first few drops were extracted after five minutes, and the fluid was then emptied into the counting chamber. It was left for three minutes to settle down. Prior to moving to a high power (10X) objective, the center big squares containing 25 smaller squares were adjusted to the light by changing to a low power (10X) objective. Four of the corner squares and one in the center had their red blood cells counted.

$$Total\ RBC\ \left(\frac{mm}{l}\right) = N\ x\frac{1}{0.2}x\frac{1}{0.1}x\ 200\ where\ N\ is\ number\ of\ cells\ counted\ , 0.1\ is\ depth\ of\ the\ chamber\ , 0.2\ is\ are\ a\ counted,\ and\ 200\ is\ dilution\ factor$$

The white blood cells count was evaluated as described by Prudent et al. (2015). A total of 380 L of diluting fluid (acetic acid, with gentian violet) were combined with 20 µl of whole blood. After removing the initial five drops of blood, the counting chamber was filled with the well-mixed, diluted blood using a pipette. For 3 min, cells were left to settle in a damp chamber. Using a low-power (10X) objective microscope, the chamber's four corners were viewed, and the number of cells in each of the four squares that had been designated as corners was counted.

$$Total\ WBC\ \left(mm^3\right) = N\ x\frac{1}{0.1}\ x\ 20\ where\ N\ is\ number\ of\ cells\ counted\ , 0.1\ is\ depth\ of\ the\ chamber\ ,\ A\ is\ area\ counted,\ 20\ is\ dilution\ factor$$

The procedures illustrated by Prudent et al. (2015) were also employed in investigating the packed cell volume (PCV). First, a heparinized capillary tube was used to collect the blood sample, and then it was cleaned and sealed with plasticine. The filled tubes were inserted into the microhaematocrit centrifuge, which was spun for 5 min at 2236 × g. Finally, the PCV was measured as a percentage by placing spun tubes on a scale that was specifically made for the purpose.

$$PVC\ (\%) = \frac{Packed\ RBC\ colum\ height}{Total\ blood\ volume\ height}x100$$

The cyanomethaglobin method described by Prudent et al. (2015) was used to measure the concentration of hemoglobin (Hb).In a test tube with 4 ml of Drabkin's solution, whole blood (20 µl) was added in a 1:250 dilution. This was thoroughly mixed, left to stand at room temperature for 10 min, and the absorbance was measured colorimetrically at 540 nm using Drabkin's solution as a control.

Statistical analysis

All experiments were performed five times ($n = 5$). The Design-Expert software version 10.0's 3-level factorial-response surface methodology (3LF-RSM) was used to optimize the tea formulation process (Statease Inc., MN, USA). The significant difference was evaluated at a 5% significant level.

Results and discussion

Model fitting and development of the optimal values for tea blends

For the various tea formulations made from the composite of *F. capensis*and *J. secunda*, the results of the 3-level factorial design-response surface technique are presented in Table 1. A range of 2200 to 7420 molTEAC/100 g of DPPH, 9.97 to 49.13 mmol Fe^{2+}/100 g of FRAP, 860 to 4278.70 mgGAE/100 g of total phenolic, 0.235 to 0.782 g/ml of packed bulk density and 1.97 to 6.62 mg/g of total dissolved solids, respectively, were observed (Table 1). With the aid of multiple linear regression analysis, quadratic models were built, and their applicability was verified at a 5% significant level. Table 2 illustrates the results of the regression analysis, which indicated that the coefficient of variation of the models ranged from 0.14 to 3.25%, and the coefficient of determination (R^2) ranged from 0.9167 to 0.9991. While the adjusted coefficient of determination (adj R^2) ranged from 0.8340 to 0.9985, the adequate precision ranged from 19.89 to 90.29. The parameters of the afore mentioned model satisfied the criteria of R^2 and adj R^2(≥ 0.80), CV($< 10\%$), and adequate precision (>4). The outcomes for the model parameters demonstrated that the models created could simulate the tea-producing process accurately.

Empirical analysis of optimized tea from *Justicia secunda* and *Ficus capensis*

The empirical expressions for the optimised tea are shown in Table 3. Each main, interactional, and quadratic significant effect was categorized into a supportingor opposing effect. The DPPH, FRAP, total phenolic, packed bulk density, and total dissolved solids were significantly ($p < 0.05$) supported by *F. capensis*. This shows that increasing the amount of *F. capensis* would improve the DPPH, FRAP, total phenolic, packed bulk density, and total dissolved

Table 3 Emperical descriptions of blood booster tea blends from *F. capensis* and *J. secunda*

Emperical Model	Main Effect		Interacton Effect		Quadratic Effect	
	Supportive	Oppositive	Supportive	Oppositive	Supportive	Oppositive
$DPPH = 5450.97 + 2148.17f + 135.33j + 407.75fj - 1062.88f^2 + 385.62j^2$	f-sig	-	fj-sig	-	-	j-sig
$FRAP = 33.41 + 12.83f + 3.33j + 6.75fj - 3.35f^2 - 3.85j^2$	f-sig j-sig	-	fj-sig	-	-	f-sig j-sig
$Totalphenoliccontent = 2203.68 + 866.33f + 688.83j + 843fj - 132.81f^2 - 190.31j^2$	f-sig j-sig	-	fj-sig	-	-	f-sig j-sig
$Packedbulkdensity = 0.59 + 0.14f + 0.097j + 0.13fj - 0.036f^2 - 0.14j^2$	f-sig j-sig	-	fj-sig	-	-	f-sig j-sig
$Totaldissolvedsolids = 4.98 + 1.58f + 0.29j + 0.74fj - 0.65f^2 + 0.37j^2$	f-sig j-sig	-	fj-sig	-	j-sig	f-sig

Sig Significant at 5% significant level, *f F. Capensis, j J. secunda, DPPH—2* 2-diphenyl-1-picrylhydrazyl, *FRAP* Ferric reducing powers

solids of the tea. Meanwhile, *J. secunda.*did not have significant ($p<0.05$) supportive effect on the DPPH. All the dependent variables were significantly ($p<0.05$) favoured by the interaction effect of *J. secunda* and *F. capensis*. Only total dissolved solids were favoured by doubling the amount of *J. secunda*in the formulation. Therefore, doubling the amount of *F. capensis* may have a detrimental impact on the tea's DPPH, FRAP, total phenolic, packed bulk density, and total dissolved solids.

The response surface contour plots for the modelling are shown in Fig. 2. The optimization showed that 52 g/100 g of *J. secunda* and 48 g/100 g of *F. capensis* were required for the development of quality tea (Table 4).

Physicochemical characteristics

Table 3 displays the findings of the physicochemical characteristics of the tea. Bulk density is linked to the financial difficulty facing the beverage sector (e.g. packaging,

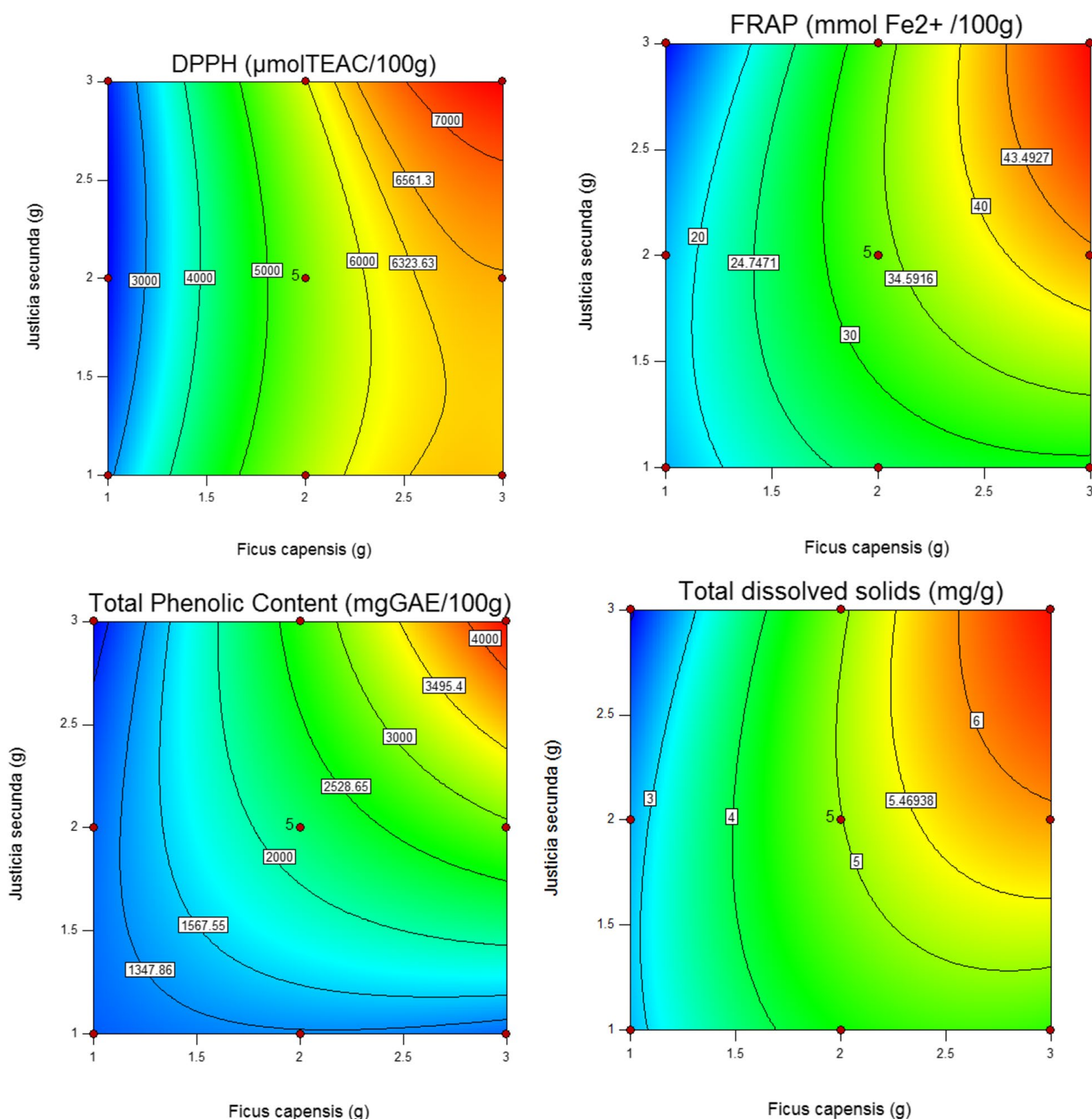


Fig. 2 Response surface contour plots for optimized blood booster tea blend from *F. capensis* and *J. secunda*

Table 4 Quality characteristics of optimized tea bends from *F. capensis* and *J. secunda*

Parameter	Value
Independent variable	
F. capensis (g/100 g)	48.00
J. secunda (g/100 g)	52.00
Physicochemical properties	
Loose bulk density (g/ml)	0.261 ± 0.01
Packed bulk density (g/ml)	0.680 ± 0.017
Porosity (%)	22.87 ± 0.10
pH	5.31 ± 0.01
Total dissolved solids (mg/g)	6.51 ± 0.11
Colour	
L (Lightness)	78.35 ± 0.42
a (Redness)	3.14 ± 0.09
b (Yellowness)	13.57 ± 0.12
Antioxidants	
DPPH (μmolTEAC/100 g)	6464.95 ± 1.97
Feric Reducing Power (mmol Fe^{2+} /100 g)	40.13 ± 0.07
Metal Chelating (%)	57.40 ± 0.62

transport, and storage costs). It is one of the critical characteristics of tea powder since it affects how easily the powder can be handled and transported. Loose bulk density (LBD) is a measurement of the amount of weight a sample can support when placed immediately on top of another. The value of the loose bulk density of optimal tea from *F. capensis* and *J. secunda*blends was 0.261 g/mL, which is the lowest density that can be achieved with compression. This score is thought to be low, which is a positive quality for packaging and storage. According to Fasuan et al. (2021), as the samples would pack better during storage without losing volume, a product with lower values of loose bulk density is preferred. Habtegebriel et al. (2018) explain that tapped bulk density is a crucial factor that affects the packaging, shipping, and marketing of dry powders. The optimal tea from *F. capensis* and *J. secunda*blends had a packed bulk density of 0.680 g/ml. It stands for the greatest compression-achievable density. Powders with a greater tapped bulk density are preferred because they require less packaging space than those with a lower tapped bulk density, which lowers the cost of shipping and packaging (Habtegebriel et al. 2018).The tea blends had a porosity of 22.87%. The fraction of void volume over total volume is known as porosity. It is typically calculated using the apparent and true densities of the material and represents a portion of the empty volume (void). The optimal tea made from a mix of *F. capensis* and *J. secunda*had a pH of 5.31. The measured pH values indicate that the designed tea belongs to the category of weak acid meals. The amount of dissolved materials in a solution is expressed by the term total dissolved solids (TDS). The total dissolved solid content yields a result of 6.51 mg/g.

The tea infusions' colour helps customers quickly judge the quality of the product and contributes to its overall aesthetic attractiveness. Therefore, when the industry grades the quality of tea-producing batches, these characteristics are taken into consideration. If the infusions are excessively pale in colour, they could be viewed as weak and of low quality. The optimized tea from *F. capensis* and *J. secunda* blends had Lightness, *L(78.35), redness, *a(3.14), and Yellowness, *b(13.57). In general, the colour of tea depends on the recipes used in the formulation, and this varies from white to green, green to different tones of brown and black (Dubey et al. 2020; Zohora & Arefin 2022). In this study, the colour was light green, which was similar to some commercial tea in the market such as lipton.

Antioxidant capacity

The scavenging of DPPH, FRAP, and metal chelating were just a few of the in vitro antioxidant assays used in this research. *F. capensis* and *J.secunda*blended optimized tea have a DPPH radical scavenging activity of 6464.95 molTEAC/100 g. The DPPH radical assay is a quick, accurate, and repeatable way to measure how well antioxidants scavenge free radicals. DPPH is a stable radical with a nitrogen center that readily forms a stable diamagnetic molecule by accepting an electron or hydrogen radical (Mandade 2011). The extract converted the DPPH radical's violet color to the non-radical DPPH-H form's yellow color (Omoregie & Okugbo 2015). These tea extracts have very high antioxidant activity when it comes to their capacity to scavenge free radicals, as measured by DPPH, and are, therefore able to stop the lipid peroxidation process. A plant extract's capacity to transport electrons is essential to the ferric reducing/antioxidant power (FRAP) assay. It evaluates how well a specific extract can convert ferric iron into ferrous iron (Igwe et al. 2022). According to the results, the tea's antioxidant capacity to reduce ferric was 40.13 mmol $Fe^{2+/}$100 g. It is remarkable that the tea extract shows high antioxidant properties. Because of this, herbal substances with strong antioxidant activity can be used to prevent and treat diseases like diabetes and cardiovascular disease that are brought on by oxidative stress (Niazmand & Razavizadeh 2021). Novel agents can be found for usage in food by doing research and development on these naturally occurring antioxidants and comparing their efficacy to synthetic agents.

By monitoring the development of ferrozine complexes with Fe^{+2} ions, the extracts' ability to chelate was determined in this research. As shown by the fact that the tea extract prevented the production of Fe^{+2} ferrozine

complexes, the tea extract chelated the iron. Itstopped the reaction from being completed, with a 57.40% success rate. The human body can produce enzyme activity thanks to the crucial role played by transition metals, which have unpaired electrons that react swiftly with peroxides to produce alkoxyl radicals. Therefore, it should be considered a crucial mechanism in the oxidation process that antioxidants chelate transition metals (Zengin et al. 2015). Furthermore, the study showed that the test tea sample has a metal chelating capacity, and this may operate as a protective mechanism against oxidative damage brought on by metal-catalyzed breakdown events (Joel et al. 2017).

Identification and quantification of phenolic compounds

The phenolic substances in the optimized functional beverage developed from extracts of *F. capensis* and *J. secunda*are listed in Table 5. The results indicated that there were high concentrations of catechin (649.50 mg/100 g), epicatechin (115.63 mg/100 g), ferulic (26.17 mg/100 g), vanillic (16.95 mg/100 g), quercetin (72.38 mg/100 g), chlorogenic acid (11.36 mg/100 g), and gallic (6.38 mg/100 g). Phenolic compounds, which belong to secondary plant metabolites, have antioxidant effects. Regular phenolic acid consumption may help lower the risk of cancer and other chronic degenerative health issues. A range of 23.1 to 66 mg/100 g of epicatechin, 6.2 to 7 mg/100 g of catechin, 17.3 to 28.8 mg/100 g of caffeic acid, 3.7 to 5.2 mg/100 g of ferulic acid, and 30.8 to 70.07 mg/100 g of gallic acid were found in fermented passion fruit beverage, according to Santo et al. (2021). One of the most effective free radical scavengers for reactive oxygen and superoxide, which are present in the body, is epicatechin. Epicatechin also reduces the possibility of low-density lipoprotein oxidation, preventing the onset of atherosclerosis (Aina et al. 2011; Lavanchy, 2011). Epicatechin has been connected to diabetics' blood glucose reduction and antiangiogenic effects. Antimicrobial, anti-inflammatory, anti-allergic, hepatoprotective, vasodilatory, and antioxidant effects have all been connected to ferulic acid (Gupta et al. 2021). According to Fernandes and Salgado (Fernandes & Salgado 2016), gallic acid has antibacterial, antioxidant, anticancer, and anti-inflammatory properties. Vanillic acid has biological actions that stimulate the immune system and have cardioprotective, neuroprotective, antioxidant, anti-inflammatory, and anti-apoptotic activities (Sharma et al. 2020).

Table 5 Phenolic Compounds of optimized tea blends from *F. capensis* and *J. secunda*(mg/100 g)

Parameter	Quantity (mg/100 g)
Epicatechin	115.63 ± 0.52
Ferulic acid	26.17 ± 0.11
Vanillic acid	16.95 ± 0.04
Gallic acid	6.38 ± 0.04
Ellaggic acid	0.27 ± 0.01
Catechin	649.50 ± 1.30
Quercetin	72.38 ± 0.73
Chlorogenic acid	11.36 ± 0.10

Hematological assay

Before the treatment, the analysis of the baseline result showed that packed cell volume (PCV), red blood cell (RBC), and hemoglobin (Hb) were not statistically ($p > 0.05$) different. In contrast, white blood cell (WBC) was significant ($p < 0.05$), as presented in Table 6. Furthermore, it was observed that after induction, apart from packed cell volume (PCV) having no significant ($p > 0.05$) difference across the groups analyzed, all other maker indices were significant ($p < 0.05$) among the groups, as shown in Table 6.

Table 6 shows the result analysis after treatment. The packed cell volume(PCV) showed a highly significant ($p < 0.05$) increase when groups A and B were compared to group C. However, group B was found to be significantly($p < 0.05$) higher compared to group A. The result analysis of the red blood cell (RBC) andhemoglobin (Hb) reveals a significant ($p < 0.05$) increases in their groups A and B when compared across group C. Meanwhile, white blood cell (WBC) of the treated groups' A and B were found to be significantly($p < 0.05$) lower compared to group C (positive control).

Table 7 shows the percentage immunity index, blood recovery index, blood recovery index score, and hematological differential index of blood-boosting potentials of optimized tea from blends of *F. capensis* and *J. Secunda*. There was no recovery for the animals feed without blood booster. All the hematological indices decreased throughout the 28 days. Samples with blood booster improved in PCV, RBC, and HN. The sample with the optimized tea was able to recover 95.28% of the PCV, 04.78% of RBC, and 97.52% of the HB. These values compared well with the 100.47%, 96.31%, and 100.39% for PCV, RBC, and HB, respectively, of the commercial blood tonic (control). The optimized tea may recover up to 100% blood if the time is extended a little. Consequently, the blood booster tea is effective as an alternative to commercial blood booster (control) and, therefore, could be recommended as a blood booster and could be commercialized. The blood recovery index score showed the recovery potential of the developed blood booster tea relative to the commercial counterpart (control). Thus, the PCV was 94.84% effective compared to chemeron, RBC was 98.40% effective, WBC

Table 6 Blood boosting potentials of optimized tea blends from *F. capensis* and *J. secunda*

Groups	Hematological Indices			
	PCV (%)	RBC (X10^6)	WBC (X10 mm^3)	HB (g/dl)
(a) Hematological profile of untreated animal				
Group A_u	42.40 ± 1.52^a	10.72 ± 0.23^a	9680.00 ± 1.06^b	10.48 ± 0.23^a
Group B_u	42.80 ± 1.92^a	10.58 ± 0.49^a	9940.00 ± 1.07^a	10.30 ± 0.26^a
Group C_u	42.40 ± 2.30^a	10.91 ± 0.50^a	9360.00 ± 1.83^c	10.24 ± 0.30^a
(b) Hematological profile of animal infected with anemia				
Group A_i	19.60 ± 0.52^a	6.44 ± 0.07^a	14960.00 ± 2.84^c	6.12 ± 0.07^a
Group B_i	20.60 ± 0.41^a	6.16 ± 0.08^c	15580.00 ± 1.80^a	5.94 ± 0.31^b
Group C_i	19.00 ± 0.58^a	6.29 ± 0.11^b	15340.00 ± 2.48^b	6.20 ± 0.04^a
(c) ***Hematological profile of animal recovered from anemia***				
Group A_t	40.40 ± 1.14^b	10.16 ± 0.72^a	10540.00 ± 2.77^b	10.22 ± 0.49^a
Group B_c	43.00 ± 0.24^a	10.19 ± 0.36^a	9460.00 ± 1.76^c	10.34 ± 0.22^a
Group C_{iu}	17.40 ± 0.67^c	5.05 ± 0.38^b	18200.00 ± 2.64^a	4.70 ± 0.33^b

Values are means ± SD (*n* = 5)

[a,b,c] Mean values with different letters as superscripts across significantlydifferent at 5% confident interval

A_u, B_u, C_u – untreated animal (initial status of animal); A_i, B_i, C_i – animal subjected to anemia; A_r – animal recovered from anemia using optimized tea from *F. capensis* and *J. secunda*, B_c—animal recovered from anemia using commercial blood tonic (control), C_{iu}—animal infected with anemia without treatment thereafter, *RBC* Red blood cell, *WBC* White blood cell, hemoglobin, *PVC* Packed cell volume

was 87.42%, and HB was 97.14%. The results showed a ranking of the effectiveness of optimized tea for the hematological indices as RBC > HB > PCV > WBC. Only white blood cells recorded below 90% recovery. The hematological differential index showed the percentage unrecoverable blood after applying the blood booster. The positive sign showed it recovered more than the onset blood level. For the PCV, only 4.72% of the initial blood was not recovered using the optimized tea, 5.22% was not recovered by the optimized blood booster, and 2.28% was not recovered for the HB. This work showed that the formulated tea blend is effective and a good alternative for the existing commercialized blood boosters.

Table 7 Blood boosting indices of optimized tea blends from *F. capensis* and *J. secunda*

Group	PCV	RBC	WBC	HB
Immunity index of uninfected animal (%)				
Group A_u	53.77	39.93	54.55	41.60
Group B_u	51.87	41.78	56.74	42.33
Group C_u	55.19	42.35	63.89	39.45
Blood recovery index of infected and then treated animal (%)				
Group A_t	95.28	94.78	95.17	97.52
Group B_t	100.47	96.31	108.88	100.39
Group C_{iu}	41.04	46.29	-5.56	45.90
Blood recovery index score for infected and then treated animal ([1] with reference to commercial blood tonic) (%)				
Group A_t	94.84	98.40	87.41	97.14
Group B_t	100	100	100	100
Group C_{iu}	40.85	48.06	-5.11	45.72
Hematological differential index (%)				
Group A_t	4.72	5.22	-8.88	2.48
Group B_t	-0.47	3.69	4.83	-0.39
Group C_{iu}	58.96	53.71	-94.44	54.10

[1] with reference to commercial blood tonic (chemeron), A_u, B_u, C_u – untreated animal (initial status of animal); A_t – animal recovered from anemia using optimized tea from *F. capensis* and *J. secunda*, B_t—animal recovered from anemia using commercial blood tonic (control), C_{iu}—animal infected with anemia without treatment thereafter; *RBC* Red blood cell, *WBC* White blood cell, hemoglobin, *PVC* Packed cell volume

Fourier transformed infrared characteristics of optimized tea from extracts of *F. capensis* and *J. secunda*

Functional groups are recognized as various constituents of a substance that regulate its chemical and biological features. A functional group may be made up of a collection of atoms in a molecule that share similar chemical properties. The bioactivity of functional beverages is dependent on the sort of functional groups present (Ertl et al. 2020; PharmaFactz 2022). The functional groups in the composite blood booster tea are listed in Table 8. The primary functional groups identified included carbamyl (amide), phenyl (phenol), carboxyl (carboxylic acid), cyano (nitrile), formyl (alkaynal), alkoxycarbonyl (ester), aryl chloride, and bromine halides. Bromo-halide, a key functional group in pipobroman, is a vital functional group in the treatment of cancer. The carboxyl group is an important functional element of bismuth subgallate, which is used to conceal the odour of flatulence. A major functional group called the cyano is present in invosidenib. Adult acute myeloid malignancy, which occurs as a result of an increase in aberrant blood cells, is treated with invosidenib. Carbamoyl is a significant functional group present in rolapitant. Rolapitant is used in cancer chemotherapy. The interferogram for the functional groups in the blood booster tea is shown in Fig. 3. The classification and distribution of functional groups in the optimized blood booster tea are presented in Table 9. The tea contained 18.75% aryl

Table 8 Functional groups optimized tea blends from *F. capensis* and *J. secunda*

S/No	Ab (cm^{-1})	T (%)	Type of Functional Group Present	Class
1	3265.15	81.14	-O–H stretch,	Phenol
			> N–H stretch	Amide
2	2922.23	82.84	-O–H stretch	Carboxylic acid
3	2851.41	85.07	-O–H stretch	Carboxylic acid
4	2370.58	93.06	-C≡N stretch	Nitrile
5	2113.40	94.32	-C≡C- stretch	Alkaynal
6	1733.21	90.95	C=O stretch	Ester
7	1602.76	74.54	C–C = C Symmetric Stretch	Alkenes
8	1524.48	80.65	N–H bend	Amide
9	1423.84	77.79	C-H bend	Alkyl
10	1319.48	79.26	CH_3 C-H bend	Ketone
11	1237.48	78.14	*N* = O Bend	Nitro
12	1028.75	59.18	> C-O stretch	Ester
13	611.28	60.71	> C–Cl stretch	Aryl halide
14	585.19	61.38	> C–Br stretch	Aryl halide
15	536.74	62.97	> C–Br stretch	Aryl halide

Ab Characteristic absorption, *T* Transmittance

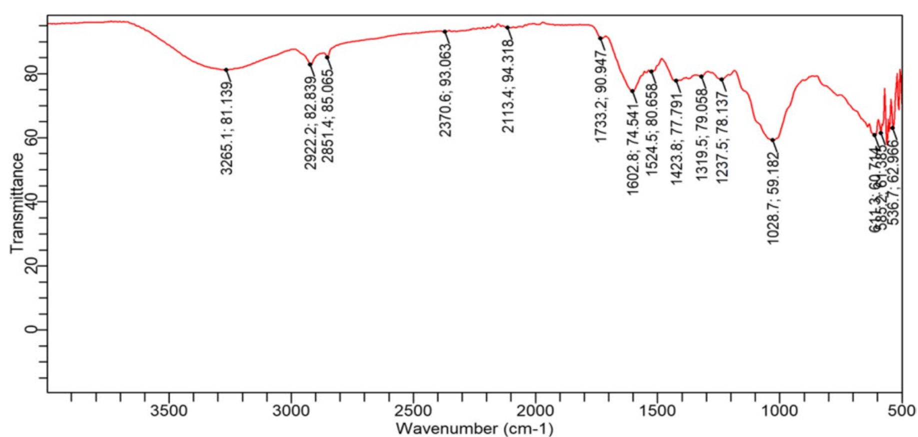


Fig. 3 Fourier transform infrared spectra of optimized blood booster tea blends from extracts of *F. capensis* and *J. secunda*

Table 9 Classification and distribution of functional groups in optimized blood booster tea blends from extracts of *F. capensis* and *J. secunda*

Class of Organic Compound	Proportion (%)
Phenol	6.25
Amide	6.25
Carboxylic acid	12.50
Nitrile	6.25
Alkaynal	6.25
Ester	12.50
Alkenes	6.25
Alkyl	6.25
Ketone	6.25
Nitro	6.25
Aryl halide	18.75

halide, 12.50% of ester, 12.50% of carboxylic acid, while each of phenol, amide, nitrile, alkaynal, alkenes, ketone, and nitro recorded 6.25%.

Conclusion

The study investigated the formulation of tea blends from *J. secunda* and *F. Capensis*; and the optimal blend of 52 g/100 g J. *secunda* and 48 g/100 g *F. capensis* were established. The tea blends have light green colour and possessed blood boosting characteristic. It is also rich in antioxidant. Moreover, it contains some phenolic substances such as epicatechin, ferulic acid, and vanillic acid, which have ability to improve healthy living and lower the risk of some chronic and degenerative health issues. The ranking of the effectiveness of the optimized tea for hematological indices was red blood cell > hemoglobin > packed cell volume > white blood cell. The tea blends could be used as a functional food ingredient and functional beverage to promotegood health.

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Authors' contributions
NNU carried out the conceptualization, methodology, investigation, validation, data curation, resources, project administration, funding acquisition, and writing manuscript. TOF carried out the conceptualization, methodology, investigation, validation, data curation, resources, project administration, data analysis, writing manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The protocols for the study was reviewed and approved by the University of Nigeria IRB, Nsukka Nigeria and informed consent was obtained from each subject prior to their participation in the study.

Consent for publication
Not applicable.

Competing interests
Nonet among authors.

Author details
[1]Department of Food Science and Technology, University of Nigeria, Nsukka, Nigeria. [2]Department of Animal Health and Production Technology, Federal College of Animal Health and Production Technology, Ibadan, Nigeria. [3]Federal College of Education Technical Asaba, Asaba, Delta State, Nigeria. [4]Department of Food Science and Technology, Federal University Oye Ekiti, Oye Ekiti, Nigeria.

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