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Production of black herbal tea from *Bacopa Floribunda*: Effect on Mineral profile, Antioxidant and Anticholinesterase properties of Bacopa Floribunda leaves

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Abstract

Bacopa floribunda is a tropical plant commonly used in folkloric medicine for the treatment of several neurodegenerative disorders, with scanty scientific basis for its use. This study sought to develop black herbal tea from *Bacopa Floribunda* leaf (BFHT) and assess the mineral profile, antinutrients, antioxidant, anticholinesterase and lipid peroxidation inhibitory properties. Freshly harvested *Bacopa floribunda* leaf (FHBF) was transformed into black herbal tea BFHT. Phytate, saponin and tannin content of *B. floribunda* leaf were significantly ($P < 0.05$) reduced after processing into BFHT. BFHT exhibited significantly ($P < 0.05$) higher total phenolics (60.08 mg GAE/g), total flavonoids (12.96 mg QE/g) and reducing power (50.38mg AAE/g). The scavenging activities of BFHT extract against DPPH and NO was significantly ($P < 0.05$) higher than that of FHBF. The inhibition capacity of BFHT (IC_{50} = 0.65 µg/ml) against Fe^{2+} induced lipid peroxidation was significantly ($P < 0.05$) higher than FHBT (IC_{50} = 0.78 µg/ml). Furthermore, BFHT (IC_{50} = 74.26 µg/ml) demonstrated significantly ($P < 0.05$) higher inhibitory capacity against acetylcholinesterase than FHBT. Thus, processing of fresh leaf of *B. floribunda* into herbal tea enhanced the nutraceutical potential of the leaf and also serves as another method of preservation.

Keywords: *B. floribunda*, mineral, antinutrient, antioxidant, acetylcholinesterase, lipid peroxidation

1. Introduction

There is increasing study on the potential role of tea especially green and black tea in the prevention and attenuation of different diseases [1]. Epidemiologically, reductions in cholesterol levels, arthritis, diabetes and osteoporosis have been linked to consumption of tea [2]. The potential role of tea especially black and green tea in prevention and attenuation of different diseases is being studied day in day out [1]. Erroneously, rooibos and herbal beverages not originated from *Camellia sinensis* are also referred to as tea, but should be accurately referred to as tisanes [1]. Herbal teas or beverages contain enormous quantity of natural bioactive compounds like phenolic acids, tannins, flavonoids, alkaloids, saponins, carotenoids and terpenoids, etc and they exhibit different biological effects when consumed, effect like antioxidant, antiaging, antibacterial, antiallergic, anticarcinogenicity and anti-inflammatory effects, etc [3].

Bacopa spp belongs to the family Scrophulariaceae with about 146 aquatic herbal species. In Ayurveda and traditional medicine, Bacopa is used as a nootropic for the improvement of intellect and memory. It is also a very important component of several Ayurvedic herbal formulations with CNS as the target and managing conditions like memory, lack of concentration, and anxiety [4]. *Bacopa spp* has been reported to have cognitive processing improvement ability, as it worked on memory by suppressing acetylcholinesterase activity [5]. Bacopa has also been implicated in Ayurvedic medicine for the treatment of inflammatory conditions like asthma and arthritis [6].

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Bacopa floribunda, one of the commonest species of Bacopa, is used for memory enhancement and retention in children and adults. It is also used in folklore for the management of cognitive dysfunction especially among the Yorubas in south western Nigeria [7]. Neurodegenerative diseases, characterized by memory loss and neuropsychiatric disorders have been reported to be treated by many traditional medicine practitioners in Nigeria using herbs like *Bacopa floribunda*, *Jatropha curcas*, *Adansonia adianthifolia* and *Talinum triangulare*. *Bacopa floribunda* has been the most prominent species that possessed potential neuroprotective and anticholinesterase activities [8]. *B. floribunda* leaf are usually freshly harvested and used immediately by the traditional medicine practitioner for the treatment of brain disorders. Previous investigation from our research group evaluated the effect of storage of *Bacopa floribunda* leaf at room temperature on antinutrient, mineral profile, HPLC phenolic fingerprinting, antioxidant and cholinergic enzyme inhibitory properties [9] [10]. However, the effect of processing of the freshly harvested leaf of *Bacopa floribunda* into herbal tea has not been explored. The aim of this study is to process/ transform *Bacopa floribunda* leaf into black tea (herbal tea) BFHT to preserve the leaf, and get this herbal leaf to many people without compromising the memory enhancing properties. The mineral profile, antinutrient, antioxidant, anticholinesterase and lipid peroxidation inhibitory capacity of the resultant tea BFHT will be determined and compared with the freshly harvested *Bacopa floribunda* leaf to see if the memory enhancing properties of the leaf is preserved.

2. Materials and Methods

2.1. Sample collection

Bacopa floribunda leaf were harvested fresh from water side area of river Oluwa in Okitipupa, Ondo state Nigeria. They were sorted, cleaned and washed to remove dirt. Sample was identified and authenticated in the University herbarium with voucher number OAUSTECH/H/720.

2.2. Sample preparation

2.2.1. Production of black tea from Bacopa floribunda leaf .One kilogram (1kg) of *Bacopa floribunda* leaf (BL) was weighed, washed and drained completely. The drained leaf was spread on a tray and allowed to wither under shade for 4 hours. The withered leaf were rolled and spread on trays for fermentation/oxidation to take place. The fermented leaf were dried at the temperature of 50°C for 6 h, cooled and milled, sieved using a standard testing sieve No.30 and packaged into an air tight container. The flow chart is presented in Figure 1. The produced tea was analyzed for mineral profile, antinutrient, antioxidant, anticholinesterase and lipid peroxidation inhibitory capacity.

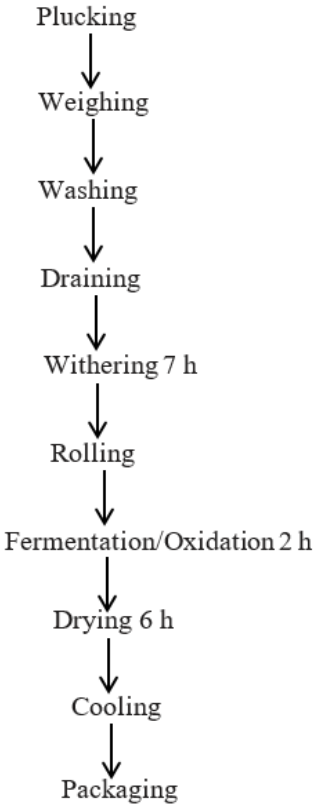

Figure 1. Production of black tea (BFHT) from *Bacopa floribunda* leaf

2.3. Antinutrient determination

Phytate content was determined according to the method described by [11]. Absorbance was measured at 500 nm using UV spectrophotometer (JENWAY 6305, Barloworld Scientific Ltd., Dunmow, Essex, UK). Phytic acid used as standard. Saponin determination was done according to the method described by [12]. This depends on colour development. Absorbance taken at 380 nm against blank. Tannin content determination was carried out according to the method of [13]. Absorbance was taken at 725 nm. Tannin was calculated using standard curve.

2.4. Mineral determination

AOAC [14] method was adopted for mineral determination. Flame photometry was used for Na and K contents determination with NaCl and KCl as standards, Vanado-molybdate method used for P.

2.5. Mineral and Molar ratio determination

The calculated Ca:P, Na:K, Ca:Mg, Ca:K, Fe:Zn mineral ratios, [K:(Ca + Mg)] milliequivalent ratio, Phytate : Ca, phytate : Zn, phytate : Fe, Ca : Phytate and [Ca] [phytate] / [Zn] molar ratios was done as described in [15] [Phytate = 660, Fe = 56, Zn = 65.40, Ca = 40].

2.6. Extract preparation

Fifty grams (50g) of the produced tea was soaked in 250 mL distilled water for 24 h, shaken intermittently. The resulting mixture was filtered using muslin cloth; rotary evaporator at 40^0C was used to concentrate the filtrate used for the analyses.

2.7. Determination of phenolic compounds

2.7.1. Total phenolic content. The method of [16] was used in determining the total phenolic content. Folin–Ciocalteu phenol reagent was used. The absorbance measured at 750 nm and the total phenolic content was reported as mg Gallic Acid Equivalents (GAE) per g.

2.7.2. Total flavonoids content. The method of [17] was used in determining the total flavonoids content. The absorbance measured at 506 nm and the total flavonoid was reported as mg quercetin equivalent per g.

2.8. Determination of Antioxidant activities

2.8.1. Reducing power. Method of [18] was used for reducing power determination. Absorbance taken at 700 nm. Ascorbic acid as standard. Increase absorbance shows increase reducing power.

2.8.2. DPPH scavenging activity. Gyamfi [19] method was used in determining DPPH scavenging activity. Absorbance was measured at 520 nm.

DPPH scavenging ability = [(Abs control – Abs sample) / (Abs control)] × 100

2.8.3. Nitric oxide (NO) radical scavenging ability. Panda [20] method was used in the determination of Nitric oxide scavenging ability. Absorbance was taken at 546 nm and inhibition percentage calculated. Control = Reaction mixture without extract.

2.9. Enzyme inhibition assay

2.9.1. Cholinesterase Activity. Perry [21] method was used for the determination of AChE activity. The substrate used for AChE activity was Acetylthiocholine iodide. Activity was measured as a change in absorbance at 412 nm for 3 min at room temperature and expressed as percentage inhibition.

2.10. Experimental animals.

Four healthy male Wistar rats (220-240) g were used for the experiment. Experimental protocols were according to revised National Institute of Health (NIH publication 1985) guidelines on handling and use of laboratory animals [22]. It was approved by the Research and Ethics Committee of Olusegun Agagu University of Science and Technology (OAUSTECH/ETHC-BCH/2020/02).

2.11. Preparation of brain homogenate

The brain tissue of the rats were removed and weighed on ice after the rats have been anesthetized and decollated using mild diethyl ether. To obtain the supernatant (SI) used for the lipid peroxidation determination, the brain tissue was homogenized with cold normal saline (1:4 w/v) on ice and centrifuged at 3,000 rpm for 10 min [23].

2.11.1. Lipid peroxidation assay. The method described in [23] using whole brain, was used for Lipid peroxidation assay. Absorbance was taken at 532 nm. Produced Malondialdehyde (MDA) was calculated and expressed as % control.

2.12. Statistical analysis.

The result was expressed as mean of three determinations. ANOVA was performed using Statistical Analysis System proprietary software (SAS version 8.3, SAS Institute Inc., Cary, NC, USA). Mean separation ($P < 0.05$) was carried out using Duncan's multiple range tests. Plotting of the graph was done using Graph pad 5.0. Linear regression analysis was used for the calculation of IC50.

3. Result

3.1. Mineral content

The production of herbal tea from *B. floribunda*, (BFHT) resulted in significant ($P < 0.05$) increase in P, K and Na content of *B. floribunda* (table 1) while there was significant ($P < 0.05$) reduction in Mg, Fe, Zn and Ca content but there was no significant

($P < 0.05$) change in Mn content of BHFT (0.19 mg/g) and freshly harvested *B. floribunda* FHBF (0.22mg/g) leaf.

3.2. Antinutrient content

The antinutrient content of herbal tea produced from *B. floribunda* leaf termed BFHT (*B. floribunda* herbal tea) is presented in Table 1. The phytate, saponin and tannin content of *B. floribunda* leaf reduced significantly ($P < 0.05$) after processing into herbal tea, phytate from 8.30mg/g FHBF to 1.18mg/g BFHT, saponin 8.37% FHBF to 1.52% BFHT and tannin 0.53% FHBF to 0.37% BFHT.

3.3. The mineral and molar ratio of minerals

Mineral and molar ratios of minerals as shown in table 2 revealed that Ca : P and Ca : K mineral ratios of *B. floribunda* significantly ($P < 0.05$) decreased after processing the leaf into herbal tea BFHT while there was no significant ($P < 0.05$) change in Na : K and Ca : Mg mineral ratios. The milliequivalent ratio of [K : (Ca + Mg)] increased significantly ($P < 0.05$) in BFHT over FHBF leaf. The molar ratios Phy:Zn and Ca:Phy of *B. floribunda* increased significantly ($P < 0.05$) in BFHT while molar ratios Phy : Zn and [Ca][Phy]/[Zn] of *B. floribunda* decreased significantly ($P < 0.05$) in BFHT.

Table 1. Mineral and antinutrient content of herbal tea from B. floribunda leaf

	FHBF	BFHT
Phosphorus (mg/g)	0.42±0.01b	1.70±0.1a
Potassium (mg/g)	7.71±0.1b	15.25±1.2a
Sodium (mg/g)	0.83±0.2b	2.00±0.01a
Calcium (mg/g)	14.81±1.1a	3.28±0.13b
Magnesium (mg/g)	7.10±0.2a	1.90±0.01b
Manganese (mg/g)	0.22±0.0a	0.19±0.0a
Iron (mg/g)	0.93±.01a	0.65±0.01b
Zinc (mg/g)	2.90±0.1a	0.16±0.0b
Phytate (mg/g)	8.30 ±0.2a	1.18±0.1b
Saponin (%)	8.37±0.12a	1.52±0.1b
Tannin (%)	0.53±0.1b	0.37±0.01b

Values = mean of three determinations ± SD. Values with the same letter on the same row are not significantly ($P < 0.05$) different. FHBF: freshly harvested *B floribunda*, BFHT: *B floribunda* herbal tea

Table 2. Mineral ratio and Molar ratio of herbal tea from *B. floribunda* leaf

Mineral ratio	**FHBF**	**BFHT**
Ca:P	35.26±1.4a	1.93±0.01b
Ca:K	1.92±0.01a	0.22±0.0b
Na:K	0.11±0.0a	0.13±0.0a
Ca:Mg	2.09±0.1a	1.73±0.1a
Fe:Zn	0.32±0.0b	4.06±0.1a
$[K:(Ca + Mg)]^X$	0.79±0.01b	6.70±0.1a
Molar ratio		
Phy : Fe	0.76±0.01a	0.15±0.0 b
Phy : Zn	0.28±0.0b	0.73±0.01a
Ca : Phy	29.38±1.3b	45.81±1.7a
$[Ca]\ [Phy]/\ [Zn]^X$	0.104±0.0a	0.059±0.01b

Values = mean of three determinations ± SD. Values with the same letter on the same row are not significantly ($P < 0.05$) different. FHBF: freshly harvested *B floribunda*, BFHT: *B floribunda* herbal tea

3.4. Total phenol, total flavonoid and reducing power capacity

The total phenol, total flavonoid and reducing power capacity of the extract of herbal tea from *B. floribunda* leaf BFHT is presented in Table 3. BFHT in comparison with FHBF leaf exhibited high and significant ($P < 0.05$) total phenolics (60.08 mg GAE/g) and total flavonoids (12.96 mg QE/g). The reducing power of BFHT (50.38 mg AAE/g) is distinctly high and significant ($P < 0.05$) than that of freshly harvested *B. floribunda* leaf (21.62 mg AAE/g).

Table 3. Total phenol, total flavonoid and reducing power of herbal tea from B. floribunda leaf

	Total phenol (mg GAE/g)	Total flavonoid (mg QE/g)	Non flavonoid (mg QE/g)	Reducing Power (mg AAE/g)
FHBF	23.58 ±1.1b	11.71 ±0.9 b	11.87 ±0.9a	21.62 ±1.1b
BFHT	60.08 ±1.15a	12.96 ±0.7a	47.12±1.11a	50.38 ±1.13a

Values = mean of three determinations ± SD. Values with the same letter on the same column are not significantly ($P < 0.05$) different. FHBF: freshly harvested *B floribunda*, BFHT: *B floribunda* herbal tea

3.5. DPPH and Nitric oxide (NO) radical scavenging abilities

The DPPH and Nitric oxide (NO) radical scavenging abilities of BFHT were assessed (Figure 2). BFHT scavenged DPPH and NO radicals in a dose dependent manner. The scavenging activities of BFHT extract against DPPH and NO was significantly ($P < 0.05$) higher than FHBF leaf (Table 4). The table revealed the IC50 values for DPPH to be 9.61 µg/ml BFHT and 10.48 µg/ml FHBF leaf. IC50 value for NO was discovered to be 10.35 µg/ml BFHT and 13.63 µg/ml FHBF leaf.

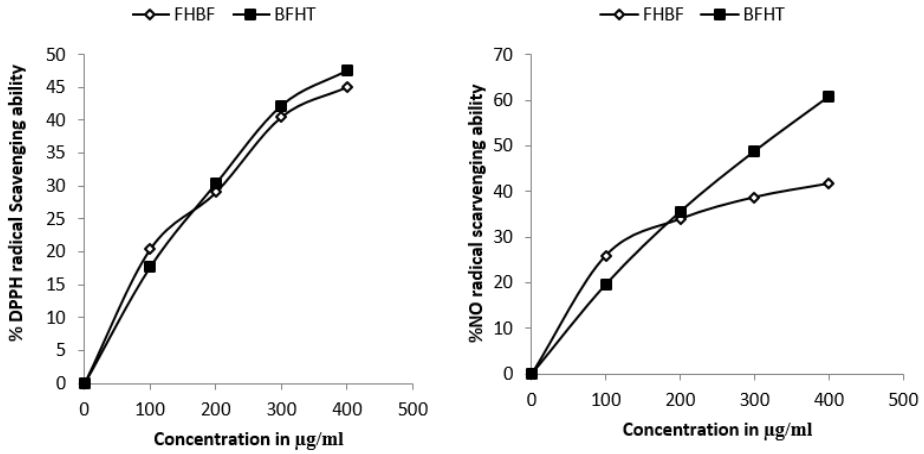

Figure 2. DPPH and NO radical scavenging ability of herbal tea from *B. floribunda* leaf
FHBF: freshly harvested B floribunda, BFHT: B floribunda herbal tea

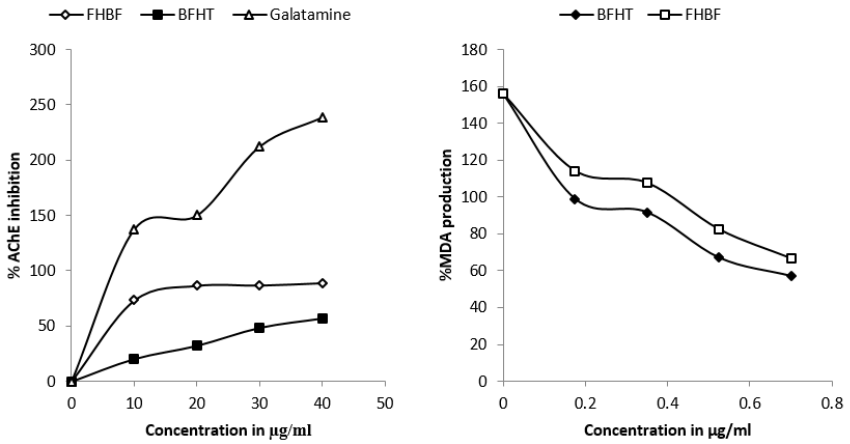

Figure 3. Inhibition of AChE activity and lipid peroxidation induced by Fe^{2+} of herbal tea from *B. floribunda* leaf
FHBF: freshly harvested *B floribunda*, BFHT: *B floribunda* herbal tea

3.6. Acetyl cholinesterase (AChE) enzyme inhibition activity

The acetyl cholinesterase enzyme inhibition activity of BFHT is presented in Figure 3. BFHT and FHBF leaf extract inhibited AChE activity as the concentration increases. IC50 values (Table 4) showed that BFHT (IC50 = 74.26 µg/ml) had a significantly ($P < 0.05$) higher inhibitory activity against AChE enzymes than FHBF leaf (IC50 = 124.88 µg/ml).

Table 4. IC50 values for DPPH, NO, Acetylcholinesterase, and inhibition of lipid peroxidation induced by Fe^{2+} of herbal tea from *B. floribunda* leaf in µg/ml

	FHBF	BFHT
DPPH	10.48 ± 0.1a	9.61 ± 0.3b
NO	13.63± 0.3 a	10.35 ± 0.1b
AChE	124.88 ± 3.8a	74.26 ± 2.5b
MDA Brain	0.78 ± 0.01a	0.65 ± 0.02b

Values = mean of three determinations ± SD. Values with the same letter on the same column are not significantly ($P < 0.05$) different. FHBF: freshly harvested *B floribunda*, BFHT: *B floribunda* herbal tea

3.7. Inhibition of Fe^{2+} induced lipid peroxidation

The lipid peroxidation ability of BFHT extracts is shown in Figure 3. The figure showed 156.19% MDA production when brain homogenates was incubated. The addition of BFHT and FHBF leaf extracts reduced the MDA content in a concentration dependent manner. Considering the IC50 values (Table 4), the inhibition capacity of BFHT (IC50 = 0.65 µg/ml) was significantly ($P < 0.05$) higher than FHBF leaf (IC50 = 0.78 µg/ml).

4. Discussion

The nutritional qualities, antinutritional factors and nutraceutical abilities of plants are often altered during the processing (fermentation) of the plants. The antinutrient contents of herbal tea produced from *B. floribunda* leaves BFHT (*B. floribunda* herbal tea) reduced significantly ($P < 0.05$) after processing into herbal tea as shown in table 1, in the process of manufacturing BFHT fermentation process was employed which could account for the reduction in these antinutrients. Fermentation is a process which includes all metabolic processes where microbial enzymes carry out oxidation, reduction, hydrolysis and other reactions [24]. Microorganisms involved in fermentation have been reported to have the ability to produce extracellular tannase enzyme which hydrolyse tannins by cleaving the ester bonds to give gallic acid and glucose thereby decreasing the tannin content. It has been reported that fermentation caused decrease in tannin content of tea dregs [24].

In the production of BFHT, the microbes involved in the fermentation might have produced an enzyme phytase which catalyses the degradation of phytates to inositol phosphates [25] resulting in the reduction of phtate content. The saponin content of trembesi leaf *Sammanea saman* reduced as a result of fermentation [26] which was similar to the result obtained in this work. The reduction in saponin could be as a result of the production of beta glucosidase by the fermentation microbe involved in BFHT production which transformed saponin to aglycones [27]; this could also reduce the bitter taste of BFHT.

Minerals are very important to a healthy diet for boosting immune system [2]. There are different schools of thought when considering mineral content of tea, there was increase in P, K and Na while decrease in Ca, Mg, Fe, and Zn content of BFHT compared to freshly harvested *B. floribunda* leaf. This result is similar to the report of [28],[2]. The increase in K and Na could be due to break down of covalent bonds found in mineral food matrix complexes during digestion [28]. Increase in P could be a result of degradation of phosphate esters in RNA contents during withering and fermentation [29]. The decrease in most minerals during tea fermentation could be due to fermentation microbe using them for energy production and enzyme activities [28].

The ratios of dietary micronutrient in nutrition research may contain more information than when concentrating on single nutrients [30]. Hence, it is more important to consider the mineral ratios than the mineral composition of foods [15]. The Ca : P ratio of BFHT and freshly harvested *B. floribunda* were greater than 1 (Table 2). Ca : P ratio greater than 1 is considered a good source of Ca [31]. The Ca : P ratios in this study could promote Ca absorption with the aim of bones and teeth formation since it is greater than 1.

The Na : K ratio were less than 1, meaning that K is greater than Na. Regular consumption of foods with large amount of K when compared with Na is beneficial for hypertensive patients [29]. Ca : Mg ratio of BFHT is less than 2, consumption of BFHT will enhance increase in Mg absorption than freshly harvested *B. floribunda* leaf with Ca : Mg ratio higher than 2.

There is decrease in Mg absorption efficiency and transformation of Mg into bones when Ca : Mg ratio is higher than 2 [15].

The Ca : K ratio reported in this work is less than 4. Ca : K ratio of 4 and above is a good source of Calcium [32]. The Fe : Zn ratio of BFHT is greater than 2 while that of freshly harvested *B. floribunda* leaf is less than 2, consumption of BFHT will encourage Zn absorption because when Fe : Zn ratio is greater than 2, Fe will not affect Zn absorption negatively [15]. Zn is important in biosynthesis of nucleic acid and proteins, it also help in phosphorus and nitrogen utilization [29]. The Milliequivalent ratios K : (Ca + Mg) of BFHT is greater than 2.2, which shows that the consumption of BFHT could encourage hypomagnesaemia in man [15].

Phytate chelates metal ions: zinc, iron, and calcium making them indigestible and unabsorbed in humans. Phytate also prevent reabsorption of zinc and calcium into the body by forming complexes with endogenously secreted zinc and calcium [33]. Phytate will impair Fe availability and absorption when phytate : Fe ratios are greater than 1.0 [15]. The phytate : Fe molar ratio of BFHT is less than 1.0 (Table 3) showing that Fe will be available for absorption when BFHT is consumed. The critical value for phytate : Zn molar ratio is 15, above this critical value, phytate will prevent Zn availability for absorption [15]. The phytate : Zn molar ratio for BFHT is less than 15, hence Zn in BFHT will be available for absorption. Phytate will not be completely precipitated if dietary Ca : phytate molar ratios are not up to 6.0 [15]. The Ca : phytate molar ratio of BFHT were more than 6.0, it means the phytate in BFHT when consumed will be completely precipitated. BFHT and freshly harvested *B. floribunda* [Ca][Phytate] / [Zn] molar ratio were less than 0.5 mol/kg, at this value calcium interference with dietary zinc availability for absorption will be zero.

Calcium will impair zinc availability for absorption when [Ca][Phy] / [Zn] molar ratio is greater than 0.5 mol/kg [34].

Plants and herbs major constituents are understandably believed to be phenolics and they are somehow linked to their various antioxidant capacities [35]. Result of table 5 indicated that there was significant increase in the content of total phenol and total flavonoid of BFHT compared to the freshly harvested *B. floribunda* leaf.

This finding was in agreement with the results of other tea researchers for instance that fermentation caused increase in the total phenolics of Malaysian herbal teas [35]. Also there was increase in the total phenol of tea leaf Chai-miang consumed in Northern Thailand [28]. In the study of the effect of fermentation on antioxidant capacity of Malaysian tea, it was found out that there was significant increase in the flavonoid contents as a result of fermentation [35]. Naturally phenolic compounds are bound with sugar in the food matrix which makes them unavailable to organism. In the process of fermentation, proteolytic enzymes like amylases, proteases and xylanases derived from fermentation microbes hydrolyse the bound phenolics into soluble-free phenols which could result in increase in the phenolics [36],[35]. Food rich in flavonoids exhibit high antioxidant activities against peroxyl radicals because of multiple hydroxyl groups of the flavonoids [37]. The increase in flavonoid content of BFHT due to fermentation could result from the increase in acidic value during fermentation thereby liberating bound flavonoid components [36]. Antioxidant compound can delay or inhibit the oxidation process of a substrate; this is done through the formation of a stable complex compound and the generation of stable antioxidant free radicals after neutralization [38]. The observed antioxidant property of BFHT was reducing power, DPPH and NO radical-scavenging ability. The ability of a substance or compound to transfer electrons is related to its reducing power and hence a significant indicator of antioxidant activity is the reducing power [39]. The reducing power as Ascorbic Acid Equivalent (AAE) (Table 3) showed that there was significant increase in the reducing power of BFHT over freshly harvested *B. floribunda* leaf. Hence, BFHT extracts possess more electrons to donate than freshly harvested *B. floribunda* that will react with free radicals, converting them to a more stable product thus terminating radical chain reactions. The observed results of reducing power in this present work is expected because reducing power ability of a food is directly proportional to its total phenol content as reported by various researchers [40].

BFHT and freshly harvested *B. floribunda* leaf extract scavenged DPPH free radicals and inhibit NO radical production from sodium nitroprusside (SNP) according to the dose of the extract used (Figure 2).

Using the IC_{50} (inhibition concentration 50) value is a better way to express the antioxidant activity (Table 4). IC_{50} is the required antioxidant concentration that will inhibit 50% of free radicals. The lower the IC_{50} value, the higher the antioxidant activity [40]. BFHT had a better radical scavenging property than freshly harvested *B. floribunda* leaf because it exhibited a lower IC 50. For DPPH scavenging ability BFHT 9.61 µg/ml and freshly harvested *B. floribunda* leaf 10.48 µg/ml while for NO scavenging ability BFHT 10.35 µg/ml and freshly harvested *B. floribunda* leaf 13.63 µg/ml. Tea has been observed to have higher antioxidant activities when compared to the fresh leaf in the study of the effect of steaming and fermentation on nutritive values, antioxidant activities, and inhibitory properties of tea leaf [28].

The termination of the role of cholinergic synapses through the inhibition of acetylcholinesterase (AChE) enzyme is one of the recent pathways in controlling AD [28]. Cholinesterase inhibition property of *B. floribunda* cannot be overlooked because of the use of *B. floribunda* in controlling neurodegenerative disorders [10]. The extract of BFHT and freshly harvested *B. floribunda* leaf inhibited AChE activities depending on the dose of the extract (Figure 3). BFHT exhibited the highest inhibitory property against AChE using IC_{50} value (Table 4). IC_{50} value of BFHT is 74.26 µg/ml and that of freshly harvested *B. floribunda* leaf is 124.88 µg/ml. This result is similar to the report of Chupeerach et al., (2021) where fermented tea leaf exhibited higher AChE inhibitions activities than fresh and steamed tea leaf. A new approach to surmount AD occurrence is the use of peptides to act as potential AChE inhibitors. These peptides form the basis of galatamine the AD synthetic drug, and can be synthesized from natural sources such as hemp seed [28]. The high AChE inhibition reported for BFHT could be due to the fermentation process employed in the production of the tea because peptides are been degraded from protein during fermentation of tea leaf.

The extracts of BFHT and freshly harvested *B. floribunda* leaf inhibited lipid peroxidation in the brain (Figure 3). IC_{50} value (Table 4) showed that BFHT have a high and significant inhibitory activity than the freshly harvested *B. floribunda* leaf.

The high inhibition capacity of BFHT extracts against Fe^{2+} induced lipid peroxidation cannot but be connected to its high phenolic and flavonoid contents which react with Fe^{2+} and prevent it from starting the lipid peroxidation chain reaction.

5. Conclusion

Findings from this study indicate that fermentation alters the nutritional qualities, antioxidant activities and anticholinesterase properties of *B. floribunda* leaf. It has also demonstrated that high quality herbal tea can be produced from *B. floribunda* leaf with interesting health beneficial potentials that can be used as functional food. The herbal tea produced from *B. floribunda* leaf BFHT had a very low amount of antinutrients: phytate, saponin and tannin compared to freshly harvested (FHBF) *B. floribunda* leaf. BFHT had the highest concentration of phytochemicals and better antioxidant activities. BFHT exhibited high inhibitory activities against AChE and lipid peroxidation in the brain. *B. floribunda* leaf could be converted to herbal tea and still perform its functions better than the fresh leaf.

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Compliance with Ethics Requirements: Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human or animal subjects (if exist) respect the specific regulation and standards.

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