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

This study investigate the effect of converting Bacopa floribunda BF leaves into Bacopa florbunda black herbal tea BFHT on the mineral profile, antinutrients, antioxidants, Anticholinesterase and lipid peroxidation inhibitory properties of Bacopa floribunda leaves.

Bacopa floribunda leaves were plucked, destalked, withered, rolled, fermented/oxidized, milled, dried, cooled and packaged. The antinutrients phytate, saponin and tannin content of B. floribunda leaves reduced significantly ($P < 0.05$) after processing into BFHT. The mineral ratios and molar ratios of minerals BFHT were within the critical values making them available for absorption. BFHT exhibited a very high and significant ($P < 0.05$) 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 Nitric oxide (NO) was significantly ($P < 0.05$) higher than freshly harvested B. floribunda leaves. BFHT (IC50 = 74.26 μg/ml) demonstrated a high significant ($P < 0.05$) inhibitory capacity against Acetyl cholinesterase enzymes. The inhibition capacity of BFHT (IC50 = 0.65 μg/ml) against Fe^{2+} induced lipid peroxidation was significantly ($P < 0.05$) higher than freshly harvested B. floribunda leaves (IC50 = 0.78 μg/ml). The study has demonstrated that high quality herbal tea BFHT could be produced from B. floribunda leaves and also serves as another method of food preservation.

Keywords: B. floribunda, BFHT, molar ratio, antioxidant, acetylcholinesterase, lipid peroxidation

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INTRODUCTION

The most consumed beverage worldwide is tea, a hot water infusion of dried, young leaves and/or buds of Camellia sinensis plant (Shannon, *et al,* 2018). Traditionally, tea consumption was for improvement of blood flow, toxin elimination and improvement in resistance to diseases (Balentine *et al*., 1997). Epidemiologically, reductions in cholesterol levels, arthritis, diabetes and osteoporosis have been linked to consumption of tea (Okafor & Ogbobe 2015). The potential role of tea especially black and green tea in prevention and attenuation of different diseases is being studied day in day out (Cleverdon *et al.,* 2018). Erroneously, rooibos and herbal beverages not originated from *Camellia sinensis* are also referred to as tea, but should be accurately referred to as tisanes (Cleverdon *et al.,* 2018). 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, anti-aging, antibacterial, anti-allergic, anti-carcinogenicity and anti-inflammatory effects, etc (Chandrasekara & Shahidi, 2018).

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, 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 (Aguiar & Borowski, 2013). *Bacopa Spp* has been reported to have cognitive processing improvement ability; it worked on memory by suppressing Acetyl cholinesterase activity (Peth-Nui *et al*., 2012). It has strong antioxidant abilities that help to prevent dementia by restoring Na^+K^+ATPase and Acetyl cholinesterase activities (Chauhan & Mehla, 2015). Bacopa has also been implicated in Ayurvedic medicine for the treatment of inflammatory conditions like asthma and arthritis (Nemetchek, *et al.,* 2016). A very important herb is *Bacopa floribunda* which 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 (Olatunji *et al.,* 2017). Neurodegenerative diseases example is memory loss and neuropsychiatric disorder 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 (Sonibare & Ayoola, 2015). *B. floribunda* leaves are usually freshly harvested and used immediately by the traditional medicine practitioner, meaning used immediately after harvest. Effect of Storage at Room Temperature on antinutrient, mineral profile, HPLC phenolic fingerprinting, antioxidant and cholinergic enzyme inhibition properties of *Bacopa floribunda* leaves has been determined (Adetuyi *et al.,* 2021, 2022). The aim of this study is to process/ transform *Bacopa floribunda* leaves into black tea (herbal tea) BFHT to preserve the leaves, to get this herbal leaves to many people and still perform its memory enhancing properties. The mineral profile, antinutrient, antioxidant, anticholinesterase and lipid peroxidation inhibitory capacity of the resultant tea BFHT be determined and compared with the freshly harvested *Bacopa floribunda* leaves to see if the memory enhancing properties of the leaves is preserved.

MATERIALS AND METHODS

Sample collection

Bacopa floribunda leaves 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.

Sample preparation

Production of black tea from *Bacopa floribunda* leaves

One kilogram (1Kg) of *Bacopa floribunda* leaves (BL) was weighed, washed and drained completely. The drained leaves was spread on a tray and allowed to wither under shade for 4hours. The withered leaves were rolled and spread on trays for fermentation/oxidation to take place. The fermented leaves 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. All analysis was in three determinations.

Antinutrient determination

Phytate content was determined according to the method described by Vaintraub and Lapteva (1988). 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 Brunner (1994). This depends on colour development. Absorbance taken at 380 nm against blank. Tannin content determination was carried out according to the method of Makkar and Goodchild (1996). Absorbance was taken at 725 nm. Tannin was calculated using standard curve.

Mineral determination

AOAC (2005) method was adopted for mineral calcium (Ca), magnesium (Mg), zinc (Zn) and iron (Fe) determination. Flame photometry was used for sodium (Na) and potassium (K) contents determination with NaCl and KCl as standards, Vanado-molybdate method used for phosphorus (P).

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 Adetuyi *et al.,* (2019) [Phytate = 660, Fe = 56, Zn = 65.40, Ca = 40].

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.

Determination of phenolic compounds

Total phenolic content

The method of Kim *et al*., (2003) 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.

Total flavonoids content

The method of Park *et al.,* (2008) 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.

Determination of Antioxidant activities

Reducing power

Method of Oyaizu (1986) was used for reducing power determination. Absorbance taken at 700 nm. Ascorbic acid as Standard. Increase absorbance shows increase reducing power.

DPPH scavenging activity

Gyamfi *et al.,* (1999) method was used in determining DPPH scavenging activity. Absorbance was measured at 520 nm.

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

Nitric oxide (NO) radical scavenging ability

Panda *et al.,* (2009) 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.

Enzyme inhibition assay

Cholinesterase Activity

Perry *et al.,* (2000) 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.

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 (NRC 2011). It was approved by the Research and Ethics Committee of Olusegun Agagu University of Science and Technology (OAUSTECH/ETHC-BCH/2020/02).

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 (Adetuyi et al 2018).

Lipid peroxidation assay

The method described in Adefegha and Oboh (2012) using whole brain, was used for Lipid peroxidation assay. Absorbance was taken at 532 nm. Produced Malondialdehyde (MDA) was calculated and expressed as % control.

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.

RESULTS

Antinutrient content

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

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* (0.22mg/g) leave.

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 leave 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 freshly harvested leave. 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.

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* leaves BFHT is presented in Table 3. BFHT in comparison with freshly harvested *B. floribunda* leaves 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 (mg AAE/g) of BFHT (50.38) is distinctly high and significant ($P < 0.05$) than that of freshly harvested *B. floribunda* leaves (21.62).

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 freshly harvested *B. floribunda* leaves (Table 4). The table revealed the IC50 values for DPPH to be 9.61 µg/ml BFHT and 10.48 µg/ml freshly harvested *B. floribunda* leaves. IC50 value for NO was discovered to be 10.35 µg/ml BFHT and 13.63 µg/ml freshly harvested *B. floribunda* leaves.

Acetyl cholinesterase (AChE) enzyme inhibition activity

The acetyl cholinesterase enzyme inhibition activity of BFHT is presented in Figure 3. BFHT and freshly harvested *B. floribunda* leaves 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 the freshly harvested *B. floribunda* leave (IC50 = 124.88 µg/ml).

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 freshly harvested *B. floribunda* leaves 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 freshly harvested *B. floribunda* leaves (IC50 = 0.78 µg/ml).

DISCUSSION

Saponins do inhibit the activities of these digestive enzymes: amylase, glucosidase, and lipase, which could result in indigestion-related diseases. The activities of enzymes responsible for protein digestion in the small intestine are always inhibited by phytate. Protein digestibility is usually affected by tannins and this can lead to reduction in the availability of essential amino acids (Samtiya *et al.,* 2020). 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 anti-nutrients. Fermentation is a process which includes all metabolic processes where microbial enzymes carry out oxidation, reduction, hydrolysis and other reactions (Tugiyanti *et al.,* 2019). 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 foods, tea dregs and fruits (Abdelrahman & Osman, 2011; Tugiyanti *et al.,* 2019; Shang *et al.,* 2019). The reduction in phytate content of BFHT as a result of fermentation is similar to the report of Kayode *et al.,* (2007) in the production of opaque sorghum beer and Lai *et al.,* (2013) in the fermentation of soymilk. 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 (Noureddini & Dang, 2009) resulting in the reduction of phtate content. The saponin content of trembesi leaves *Sammanea saman* and soymilk reduced as a result of fermentation (Sariri *et al.,* 2018; Lai *et al.,* 2013) 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 (Qian *et al.,* 2018) this could also reduce the bitter taste of BFHT.

Minerals are very important to a healthy diet for boosting immune system (Okafor & Ogbobe, 2015). 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* leave. This result is similar to the report of Chupeerach *et al.,* (2021) and Okafor and Ogbobe, (2015). In the study of lemon balm and sage herbal teas, it was reported that the minerals reduced all through in the lemon balm and sage herbal teas from their fresh leaves (Yaman, 2020). The increase in K and Na could be due to break down of covalent bonds found in mineral food matrix complexes during digestion (Chupeerach *et al.,* 2021). Increase in P could be a result of degradation of phosphate esters in RNA contents during withering and fermentation (Jabeen *et al.,* 2019). The decrease in most minerals during tea fermentation could be due to fermentation microbe using them for energy production and enzyme activities (Chupeerach *et al.,* 2021).

Using the ratios of dietary micronutrient in nutrition research may contain more information than when concentrating on single nutrients (Kelly *et al.,* 2018). Hence it is more important to look at the mineral ratios than the mineral composition of foods (Adetuyi *et al.,* 2019). 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 (Alinnor & Oze, 2011). 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 (Jabeen *et al.,* 2019). Ca : Mg ratio of BFHT is less than 2, consumption of BFHT will enhance increase in Mg absorption than freshly harvested *B. floribunda* leaves 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 (Mai *et al.,* 2003).

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 (Watts, 2010). The Fe : Zn ratio of BFHT is greater than 2 while that of freshly harvested *B. floribunda* leaves 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 (Pérès *et al*., 2001). Zn is important in biosynthesis of nucleic acid and proteins, it also help in phosphorus and nitrogen utilization (Jabeen et al., 2019). 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 (NRC, 1989).

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 (Gibson *et al.,* 2010). Phytate will impair Fe availability and absorption when phytate : Fe ratios are greater than 1.0 (Hurrell *et* *al.,* 2003). 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 (Adetuyi *et al.,* 2021). 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 (Adetuyi *et al.,* 2011). 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 (Akindahunsi & Oboh, 1999).

Plants and herbs major constituents are understandably believed to be phenolics and they are somehow linked to their various antioxidant capacities (Ibrahim *et al.,* 2014). Usually flavonoids are regarded as antioxidant molecules that could cause reduction in the effect of cellular oxidative stress (Ojo *et al.,* 2018). Result of table 3 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 and *Echinacea* spp, a medicinal herb consumed as herbal drink in the Western countries (Ibrahim *et al.,* 2014; Rizzello, 2013). Also there was increase in the total phenol of tea leaves Chai-miang consumed in Northern Thailand (Chupeerach *et al.,* 2021). 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 (Ibrahim *et al.,* 2014). 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 (Adetuyi and Ibrahim, 2014; Ibrahim *et al.,* 2014). Food rich in flavonoids exhibit high antioxidant activities against peroxyl radicals because of multiple hydroxyl groups of the flavonoids (Yashin, *et al.,* 2017). 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 (Adetuyi & Ibrahim, 2014). 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 (Rahman *et al.,* 2021). 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 (Ayoola *et al.,* 2019). 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 (Wijayanti *et al.,* 2017). The higher the polyphenolic content the higher the reducing power (Lee *et al.*, 2007).

BFHT and freshly harvested *B. floribunda* leave 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 IC50 (inhibition concentration 50) value is a better way to express the antioxidant activity (Table 4). IC50 is the required antioxidant concentration that will inhibit 50% of free radicals. The lower the IC50 value, the higher the antioxidant activity (Wijayanti *et al.,* 2017). BFHT had a better radical scavenging property than freshly harvested *B. floribunda* leave because it exhibited a lower IC 50. For DPPH scavenging ability BFHT 9.61 µg/ml and freshly harvested *B. floribunda* leave 10.48 µg/ml while for NO scavenging ability BFHT 10.35 µg/ml and freshly harvested *B. floribunda* leave 13.63 µg/ml. The pathological intermediary in reactions leading to some neurodegenerative diseases like AD, Parkinson's disease etc is the endogenously generation of Nitric oxide (NO) radicals (Sumanont *et al.*, 2004). Tea has been observed to have higher antioxidant activities when compared to the fresh leaves in the study of the effect of steaming and fermentation on nutritive values, antioxidant activities, and inhibitory properties of tea leaves (Chupeerach *et al.,* 2021)

The termination of the role of cholinergic synapses through the inhibition of acetylcholinesterase (AChE) enzyme is one of the recent pathways in controlling AD (Chupeerach *et al.,* 2021). Cholinesterase inhibition property of *B. floribunda* cannot be overlooked because of the use of *B. floribunda* in controlling neurodegenerative disorders (Adetuyi *et al.,* 2022). The extract of BFHT and freshly harvested *B. floribunda* leave inhibited AChE activities depending on the dose of the extract (Figure 3). BFHT exhibited the highest inhibitory property against AChE using IC50 value (Table 4). IC50 value of BFHT is 74.26 µg/ml and that of freshly harvested *B. floribunda* leave is 124.88 µg/ml. This result is similar to the report of Chupeerach *et al.,* (2021) where fermented tea leaves exhibited higher AChE inhibitions activities than fresh and steamed tea leaves. 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 (Chupeerach *et al.,* 2021). 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 leaves. The extracts of BFHT and freshly harvested *B. floribunda* leaves inhibited lipid peroxidation in the brain (Figure 3). IC50 value (Table 4) showed that BFHT have a high and significant inhibitory activity than the freshly harvested *B. floribunda* leaves. 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.

CONCLUSION

This study has provided useful information on the mineral profile, antioxidant and anticholinesterase qualities of *B. floribunda* and the health benefit that could be derived from the consumption of *B. floribunda* leaves. The study has also demonstrated that high quality herbal tea can be produced from *B. floribunda* leaves and the knowledge from this work would encourage the production of herbal tea as another method of food preservation. The herbal tea produced from *B. floribunda* leaves BFHT had a very low amount of antinutrients: phytate, saponin and tannin compared to freshly harvested *B. floribunda* leaves. 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* leaves could be converted to herbal tea and still perform its functions better than the fresh leaves.

REFERENCES

Abdel Rahman I.E & Osman M.A (2011). Effect of sorghum type (*Sorghum bicolor*) and traditional fermentation on tannins and phytic acid contents and trypsin inhibitor activity. *Journal of Food, Agriculture and Environment* 9 (3&4), 163-166.

Adefegha, S.A., & Oboh, G. (2012). Inhibition of key enzymes linked to type 2 diabetes and sodium nitroprusside-induced lipid peroxidation in rat pancreas by water extractable phytochemicals from some tropical spices. *Pharmaceutical Biology,* 50 (7), 857– 865.

Adetuyi, F.O., Karigidi, K.O., Akintimehin, E.S., & Adeyemo, O.N. (2018) Antioxidant Properties of *Ageratum Conyzoides* L. Asteraceae Leaves. *Bangladesh Journal of Science and Industrial Research* 53(4), 265–276. DOI: 10.3329/bjsir.v53i4.39190.

Adetuyi, F.O., & Ibrahim, T.A. (2014) Effect of Fermentation Time on the Phenolic, Flavonoid and Vitamin C Contents and Antioxidant Activities of Okra (*Abelmoschus esculentus*) Seeds. *Nigeria Food Journal* 32(2), 128 -137

Adetuyi, F.O., Akintimehin, E.S., & Karigidi, K. O. (2021) Effect of Storage at Room Temperature on Antinutrient and Mineral Profile of *Bacopa floribunda* leaves. *Coast Journal of the School of Science* (OAUSTECH) 3(2), 665 - 672.

Adetuyi F.O., Osagie, A.U., & Adekunle, A.T. (2011). Nutrient, antinutrient, mineral and zinc bioavailability of okra *Abelmoschus esculentus* (L) Moench Variety. *American Journal of Food and Nutrition.* 1(2), 4 9 - 5 4 . doi:10.5251/ajfn.2011.1.2.49.54.

Adetuyi F.O., Karigidi, K.O. Akintimehin, E.S., & Fajembola, T.F. (2019). Effect of postharvest UV-C irradiation as physical elicitor on anti-nutritional factor, B-vitamins and mineral profile of *Clerodendrum volubile* leaves. *Croatian Journal of Food Technology, Biotechnology and Nutrition.* 14 (3-4), 113 – 120

Adetuyi, F.O., Akintimehin, E.S., & Karigidi K.O. (2022) Comparative analysis of freshly harvested and stored *Bacopa floribunda* leaves: HPLC phenolic fingerprinting, antioxidant and cholinergic enzyme inhibition properties. *Advances in Traditional Medicine* https://doi.org/10.1007/s13596-021-00626-y

Ayoola, M.B., Ejiofor, N.C., & Ezeagu, I.E. (2019) In vitro-evaluation of the antioxidant properties of *Moringa oleifera* and *Camelia sinensis* leaves. *Advances in Food Technology and Nutritional Science.* 5(1), 13-18. doi: 10.17140/AFTNSOJ-5-152

Akindahunsi A.A., & Oboh G. (1999). Effect of some post-harvest treatments on the bioavailability of zinc from some selected tropical vegetables. *La Rivista Italiana Delle Grasse.* 76, 285-287.

Aguiar, S., & Borowski,T. (2013). Neuropharmacological review of the nootropic herb *Bacopa monnieri. Rejuvenation Research.*16, 313–326.

Alinnor, I.J., & Oze, R. (2011). Chemical evaluation of the nutritive value of *Pentaclethra macrophylla* benth (African Oil Bean) Seeds. *Pakistan Journal of Nutrition* 10(4), 355-359.

AOAC (2005). Association of official analytical chemists, official methods of analysis (18th ed.). Washington, DC: AOAC International.

Balentine, D.A., Wiseman, S.A., & Bouwens, L.C. (1997) The chemistry of tea flavonoids. *Critical Review in Food Science and Nutrition.* 37, 693–704

Brunner, J.H., (1984). Direct spectrophotometric determination of saponin. *Analytical Chemistry* 34, 1314 -1326.

Chandrasekara, A., & Shahidi, F (2018) Herbal beverages: Bioactive compounds and their role in disease risk reduction - A review. *Journal of Traditional and Complementary Medicine* 8, 451 – 458

Chauhan, N.B., & Mehla, J. (2015) Bioactive nutraceuticals and dietary supplements in neurological and brain disease - Ameliorative effects of nutraceuticals in neurological disorders 2015 Elsevier Inc. Chapter 27. http://dx.doi.org/10.1016/B978-0-12-411462-3.00027-8

Chupeerach, C., Aursalung, A., Watcharachaisoponsiri, T., Whanmek, K., Thiyajai, P., Yosphan, K., et al (2021) The Effect of Steaming and Fermentation on Nutritive Values, Antioxidant Activities, and Inhibitory Properties of Tea Leaves. *Foods* 10, 117. https://doi.org/10.3390/ foods10010117

Cleverdon, R., Elhalaby, Y., McAlpine, M.D., Gittings, W & Ward, W.E (2018) Total Polyphenol Content and Antioxidant Capacity of Tea Bags: Comparison of Black, Green, Red Rooibos, Chamomile and Peppermint over Different Steep Times. *Beverages* 4(15), 1 - 13 doi:10.3390/beverages4010015

Gibson, R.S., Bailey, K.B., Gibbs, M., & Ferguson, E.L (2010) A review of phytate, iron, zinc, and calcium concentrations in plant-based complementary foods used in low-income countries and implications for bioavailability. *Food and Nutrition Bulletin*, 31(2), s134 – s146

Gyamfi, M.A., Yonamine, M., & Aniya, Y. (1999). Free radical scavenging action of medicinal herbs from Ghana: thonningia sanguine on experimentally induced liver injuries. *General Pharmacology*, 32 (6), 661–667.

Hurrell, R. F., Juillerat, M.A., Reddy, M.B., Lynch, S.R., Dassenko, S.A., & Cook. J.D. (2003). Soy protein, phytate, and iron absorption in humans. *The American Journal of clinical nutrition*, 56(3), 573-578.

Ibrahim, N. A., Mustafa, S., & Ismail, A. (2014) Effect of lactic fermentation on the antioxidant capacity of Malaysian herbal teas. *International Food Research Journal* 21(4), 1483-1488

Jabeen, S., Alam, S., Saleem, M., Ahmad, W., Bibi, R., Hamid, F.S et al (2019) Withering timings affect the total free amino acids and mineral contents of tea leaves during black tea manufacturing. *Arabian Journal of Chemistry* 12, 2411–2417

Kayode, A. P., Hounhougigan, J. D. & Nout, M. J. (2007). Impact of brewing process operation on phytate, phenolic compounds and *in vitro* solubility of iron and zinc in opaque sorghum beer. *LWT food science and technology* 40: 834 - 841.

Kelly, O.J., Gilman, J.C., & Ilich, J.Z (2018). Utilizing Dietary Micronutrient Ratios in Nutritional Research May be More Informative than Focusing on Single Nutrients. *Nutrients* 10(107), 1 - 24 doi:10.3390/nu10010107

Kim, D.O., Chun, O.K., Kim, Y.J., Moon, H.Y., & Lee, C.Y. (2003). Quantification of polyphenolics and their antioxidant capacity in fresh plums. *Journal of Agricultural and Food Chemistry,* 516, 509-6515.

Lai, L., Hsieh, S., Huang, H., & Chou, C (2013). Effect of lactic fermentation on the total phenolic, saponin and phytic acid contents as well as anti-colon cancer cell proliferation activity of soymilk. *Journal of Bioscience and Bioengineering* 115 (5), 552 – 556

Lee, Y.R., Woo, K.S., Kim, K.J., Son, J-R. & Jeong, H-S. (2007). Antioxidant activities of ethanol extracts from germinated Specialty Rough Rice. *Food Science Biotechnology* 16(5), 765 – 770.

Makkar, A.O.S., & Goodchild, V.A. (1996) Quantification of tannin: A laboratory manual. International centre for Agricultural Research in the Dry area (ICARDA). Allepo, Syria iv, 25.

Mai, T.T., Hung, N.K., Kawakami, M., Kawase, M., & Chuyen. N. (2003). Micronutrient status of primary school girls in rural and urban areas of South Vietnam. *Asia Pacific Journal of Clinical Nutrition.* 12 (2), 178-185.

Nemetchek, M.D., Stierle, A.S., Stierle, D.B., & Lurie, D (2016) The Ayurvedicplant *Bacopa monnieri* inhibits inflammatorypathways in the brain. *Journal of Ethnopharmacology* http://dx.doi.org/10.1016/j.jep.2016.07.073

Noureddini, H., & Dang, J. (2009) Degradation of phytates in distiller's grains and corn gluten feed by Aspergillus niger phytase. *Applied Biochemistry and Biotechnology* 159, 11 - 23.

National Research Council (US) (2011) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Guide for the Care and Use of Laboratory Animals. 8th edition. Washington DC: National Academies Press (US).

National Research Council (NRC) (1989) Food and Nutrition Board Recommended Dietary Allowances. 10th Edition, National Academy Press, Washington DC, 82.

Ojo, O.A., Ojo, A.B., Ajiboye, B.O., Oyinloye, B.E., Akinyemi, A.J., Okesola, M.A., et al (2018). Chromatographic fingerprint analysis, antioxidant properties, and inhibition of cholinergic enzymes (acetylcholinesterase and butyrylcholinesterase) of phenolic extracts from *Irvingia gabonensis* (Aubry-Lecomte ex O'Rorke) Baill bark. *Journal of Basic and Clinical Physiology and Pharmacology*, 29(2), 217–224 https://doi.org/10.1515/jbcpp-2017-0063

Okafor, G.I., & Ogbobe, N.M (2015) Production and Quality Evaluation of Green and Black Herbal Teas from *Moringa oleifera* Leaf. *Journal of Food Resource Science* 1 – 11. DOI: 10.3923/jfrs.2015.

Olatunji B.P, Fasola, T.R., Onasanwo, S.A., Akinyemi, A.J., Adeniyi P.A., & Ishola. A.O. (2017). Neuronal Alterations and Antioxidant Status of Lipopolysaccharide Induced Neuronal damage in Mice: Efficacy of Three Medicinal Plants. *Journal of Applied Pharmaceutical Science* 7 (12), 156-162.

Oyaizu, M. (1986) Studies on products of browning reactions: Antioxidant activities of products of browning reaction prepared from glucose amine. *Japanese Journal of Nutrition* 44, 307-315.

Panda, B.N., Raj, A.B., Shrivastava, N.R. & Prathani, A.R. (2009) "The evaluation of nitric oxide scavenging activity of *Acalypha indica* Linn Root," *Asian Journal of Research in Chemistry,* 2(2), 148–150.

Park, Y-S., Jung, S-T., Kang, S-G., Heo, B.K., Arancibia-Avila, P., Toledo, F., et al (2008) Antioxidants and proteins in ethylene-treated kiwifruits. *Food Chemistry,* .107, 640–648

Pérès, J., Bureau, F., Neuville, D., Arhan, P., & Bouglé. D. (2001). Inhibition of zinc absorption by iron depends on their ratio. *Journal of Trace Elements in Medical Biology.* 15(4), 237-241 DOI: 10.1016/S0946-672X (01)80039-0

Perez, V. & Chang. E.T. (2014). Sodium-to-potassium ratio and blood pressure, hypertension, and related factors. Advances in Nutrition: *Advance Nutrition* 5(6), 712-741.

Peth-Nui, T., Wattanathorn, J., Muchimapura, S., Tong-Un, T., & Piyavhatkul, N. (2012) Effects of 12-week *Bacopa monnieri* consumption on attention, cognitive processing, working memory, and functions of both cholinergic and monoaminergic systems in healthy elderly volunteers. *Evidence-Based Complementary Alternative Medicine,* 1-10, doi:10.1155/2012/606424.

Qian, B., Yin, L., Yao, X., Zhong, Y., Gui, J., Lu, F., et al (2018) Effects of fermentation on the hemolytic activity and degradation of *Camellia oleifera* saponins by *Lactobacillus crustorum* and *Bacillus subtilis FEMS Microbiology Letters*, 365 (7), 1 – 7. doi: 10.1093/femsle/fny014

Rahman, M., Jahan, I.A., Ahmed, S., Ahmed, K.S., Roy, M., Zzaman, W. et al (2021) Bioactive compounds and antioxidant activity of black and green tea available in Bangladesh. *Food Research* 5 (3), 107 – 111

Rizzello, C. G., & Coda, R. (2013). Lactic fermentation as a tool to enhance the functional features of *Echinacea* spp. *Microbial Cell Factory* 12 (44), 1-15.

Sariri, A.K., Mulyono, A.M.W., & Tari, A.I.N (2018) The utilization of microbes as a fermentation agent to reduce saponin in Trembesi leaves (*Sammanea saman*). IOP Conf. Series: *Earth and Environmental Science* 142, 1 – 5 doi :10.1088/1755-1315/142/1/012041

Samtiya, M., Aluko, R.E., & Dhewa, T (2020) Plant food anti-nutritional factors and their reduction strategies: an overview. *Food Production, Processing and Nutrition* 2(6), 1 – 14 https://doi.org/10.1186/s43014-020-0020-5

Shang, Y.F., Cao, H., Ma, Y.L., Zhang, C., Ma, F., Wang, C.X. et al (2019) Effect of lactic acid bacteria fermentation on tannins removal in Xuan Mugua fruits. *Food Chemistry* 274, 118–122.

Shannon, E., Jaiswal, A.K., & Abu-Ghannam, N. (2018) Polyphenolic content and antioxidant capacity of white, green, black, and herbal teas: a kinetic study. *Food Research* 2 (1), 1 – 11

Sonibare M.A., & Ayoola, I.O. (2015). Medicinal plants used in the treatment of neurodegenerative disorders in some parts of Southwest Nigeria. *African Journal of Pharmacy and Pharmacology,* 9(38), 956-965, DOI: 10.5897/AJPP2014.4164.

Sumanont, Y., Murakami, Y., Tohda, M., Vajragupta, O., Matsumoto, K., & Watanabe H (2004). Evaluation of the nitric oxide radical scavenging activity of manganese complexes of curcumin and its derivative. *Biological and Pharmaceutical Bulletin,* 27(2), 170—173.

Tugiyanti, E., Susanti, E., & Sulistyawan, I. H (2019) Effect of Tea Dregs Form and Different Fermentation Process on the Nutrient, Tannin, Saponin, flavonoid content and Antioxidant Activity. *Pakistan Journal of Nutrition* 18(1), 25 – 33 DOI: 10.3923/pjn.2019.25.33

Vaintraub, I.A., & Lapteva, N.A. (1988). "Colorimetric determination of phytate in unpurified extracts of seeds and the products of their processing," *Analytical Biochem*istry 175 (1), 227–230.

Watts, D. L. (2010). HTMA Mineral Ratios. A brief discussion of their clinical importance. *Trace Elem Newsletter.* 21, 1-3.

Wijayanti, E.D., Setiawan, N.E ., & Cristi, J.P (2017) Effect of Lactic Acid Fermentation on Total Phenolic Content and Antioxidant Activity of Fig Fruit Juice (Ficus carica) *Advances in Health Sciences Research* 2, 282 -289

Yaman, C (2020). Lemon balm and sage herbal teas: Quantity and infusion time on the benefit of the content. *Ciência e Agrotecnologia,* 44, 1 – 11 http://dx.doi.org/10.1590/1413-7054202044023220

Yashin, A., Yashin, Y., Xia, X., & Nemzer, B. (2017). Antioxidant activity of spices and their impact on human health: A review. *Antioxidants*, 6 (70), 1–18. doi:10.3390/antiox6030070

APPENDICES

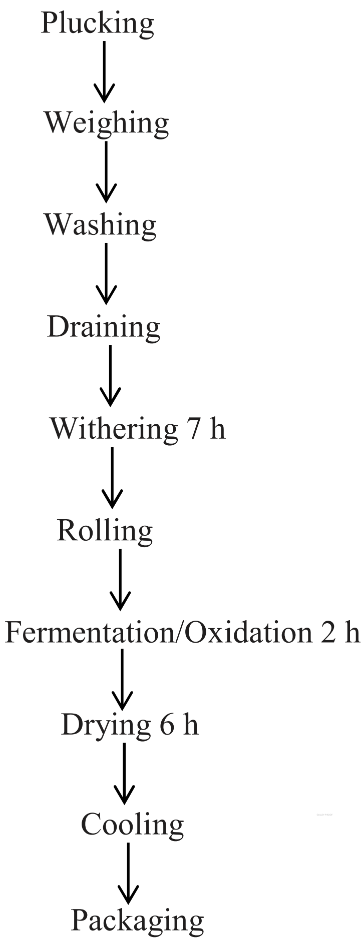


Figure 1:

Table 1: Mineral and antinutrient content of herbal tea from *B. floribunda* leaves

Mineral/Antinutrient	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* leaves

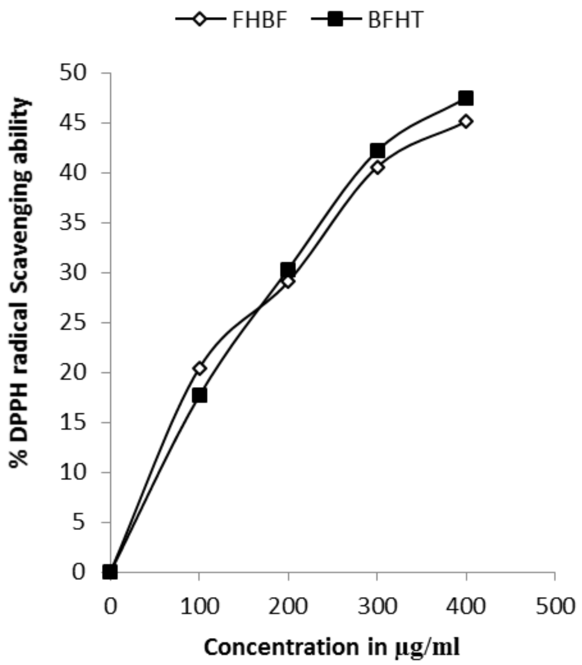
Mineral ratio	FHBF	BFHT
Ca:P	35.26±1.4a	1.93±0.01b
Ca:K	1.92±0.01a	0.22±0.0b
Na:K	0.11±0.0a	0.13±0.0a
Ca:Mg	2.09±0.1a	1.73±0.1a
Fe:Zn	0.32±0.0b	4.06±0.1a
[K:(Ca + Mg)]^X	0.79±0.01b	6.70±0.1a
Molar ratio		
Phy : Fe	0.76±0.01a	0.15±0.0 b
Phy : Zn	0.28±0.0b	0.73±0.01a
Ca : Phy	29.38±1.3b	45.81±1.7a
[Ca]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

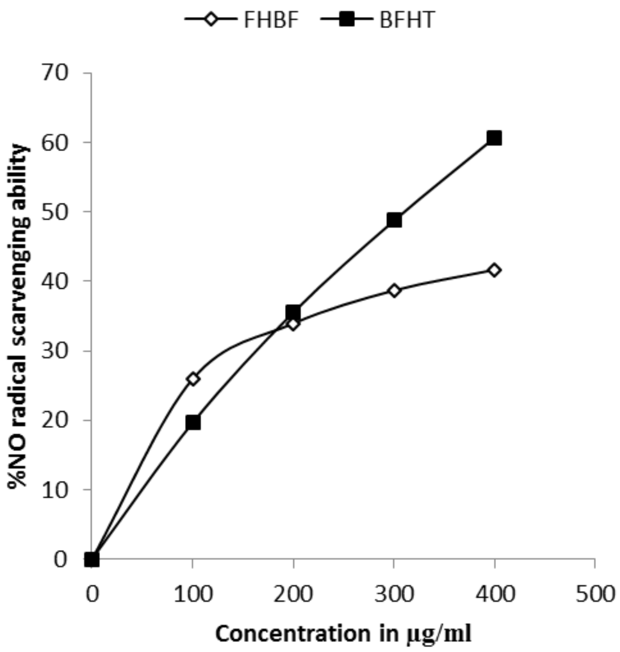
Values = mean of three determinations ± SD. Values with the same letter on the same row are not significantly ($P < 0.05$) different. FHBF: freshly harvested *B floribunda*, BFHT: *B floribunda* herbal tea

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

	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


FHBF
BFHT
% DPPH radical Scavenging ability
50
45
40
35
30
25
20
15
10
5
0
0
100
200
300
400
500
Concentration in µg/ml


FHBF
BFHT
%NO radical scarvenging ability
70
60
50
40
30
20
10
0
0
100
200
300
400
500
Concentration in µg/ml

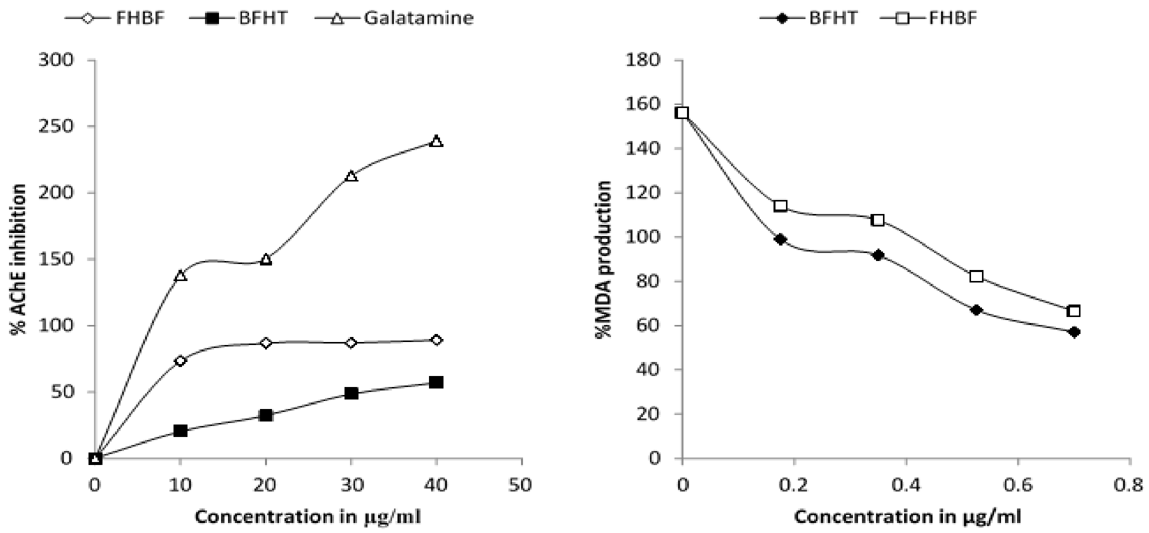


Fig. 3 Inhibition of AChE activity and lipid peroxidation induced by Fe^{2+} of herbal tea from *B. floribunda* leaves

FHBF: freshly harvested *B floribunda*, BFHT: *B floribunda* herbal tea

Table 4: IC$_{50}$ values for DPPH, NO, Acetylcholinesterase, and inhibition of lipid peroxidation induced by Fe^{2+} of herbal tea from *B. floribunda* leaves 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