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Antioxidant activity study and GC-MS Profiling of Bamboo Seed Variety

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ABSTRACT

Extensive research has been conducted on the antioxidant properties of bamboo seed extract, particularly from the Bamboo (*subfamily Bambusoideae*) Linn plant. Due to their high antioxidant content, bamboo seeds have garnered interest for their potential health benefits in combating age-related chronic illnesses such as diabetes, cancer, Alzheimer's, Parkinson's, and cardiovascular disease. The consumption of bamboo-derived products on a daily basis may play a role in reducing the risk of developing these conditions. Antioxidants play a crucial role in the food and pharmaceutical industries, as they help counteract free radicals that can degrade products during processing and storage. The chemical components of the ethanol extract of Bamboo seed variety were analyzed using Gas Chromatography-Mass Spectrometry (GC-MS), identifying twenty-four compounds. These included compounds such as 9,12-Octadecadienoic acid (Z,Z)-, Hexadecanoic acid ethyl ester, and Tetradecanoic acid -12-methyl- methyl ester, which were found in both *Bambusa arundinacea* and *Dentrocalamus strictus*. Furthermore, Linoleic acid ethyl ester and Octadecanoic acid were predominant in *Bambusa arundinacea*, while (E)-9-Octadecenoic acid ethyl ester, n-Hexadecanoicacid, Oleic Acid, and 12-Methyl-E,E-2,13-octadecadien-1-ol were found in *Dentrocalamus strictus* only. Other phytonutrients were also present in bamboo seed varieties, indicating their potential use in the treatment of various diseases as antioxidants and as a viable rice substitute.

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1- Introduction

Grass plants classified as bamboos belong to the Bambusoideae subfamily and Poaceae family. They are predominantly found in tropical and subtropical regions worldwide and are known for their high sustainability [1,2]. Globally, there are around 121 genera and approximately 1662 species of bamboo [1], with about 31.5 million hectares of bamboo plantations [3]. The highest diversity of bamboo species is concentrated in Asia, closely followed by South America and Africa [1,4]. Brazil boasts a vast natural bamboo collection, with over 200 species spread across 180,000 km^2 [4,5].

Due to its versatility across various fields, bamboo cultivation has gained international acclaim [6]. For instance, bamboo can be utilized in furniture production by flattening culms into boards [7] and in the construction sector, where leaves and ashes can be used as a supplement to cement [5]. Moreover, bamboo finds applications in the pharmaceutical industry, food sector [8], handicrafts [9], cycling frames, and medicines.

Notably, bamboo fibers are marketed as Jelucel® BF, Nutriloid® Bamboo Fiber, and CreaFibe in various countries [4]. Additionally, the Ministry of Health in the People's Republic of China highlights the use of bamboo as an additive in food and pharmaceutical products [10].

Bamboo leaves are rich in protein, calcium, iron, and magnesium, while being low in thein and caffeine. They have been employed in fortifying different food items biologically to enhance their nutritional content, reduce harmful acrylamide formed during processing, or extend shelf-life and enhance flavor [6,11–13]. These leaves can serve as food additives, flavorings, and preservatives in various food categories.

The potential of bamboo has attracted interest from the food, nutraceutical, cosmeceutical, and pharmaceutical industries. This paper aims to explore the antioxidant properties of bamboo and conduct a Fatty acid analysis of a bamboo seed variety isolated from the ethanolic extract using Gas Chromatography (GC) with Mass Spectrometry (MS).

2.Materials and Methods

1.1.Plant specimen and collection

Bamboo seed is an underutilized species in India. The bamboo seed varieties of (*Bambusa arundinacea* and *Dentrocalamus strictus*) grown in Kerala were obtained from the Department of Forest Service in Salem, ground into flour, and used for analysis. The analytical grade chemicals and solvents used in this study were sourced from Sigma Aldrich and E. Merck in Germany.

1.2.Preparation of plant materials

The seeds were carefully dried in a controlled environment to prevent direct sunlight exposure, cleaned, and manually winnowed to eliminate dust and other foreign particles before being used for additional analysis. The selected varieties of bamboo paddy were authenticated by Prof. P. Jayaraman, Director of the Institute of Herbal Botany, Plant Anatomy Research Center, in Chennai, Tamil Nadu.

1.3.Antioxidant Activity evalution

1.3.1. 2,2-diphenyl-1-picrylhydrazyl Radical Scavenging Activity (DPPH)

The antioxidant activity of the bamboo kernel flour (Ba-T, Ba-K, Ds-T and Ds-K)

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was determined in terms of hydrogen donating or radical scavenging ability, using the stable radical DPPH, according to the method of [14]. The sample extracts at various concentrations (20 - 100 µg) was taken and the volume was adjusted to 100 µl with methanol. 5 ml of 0.1 mM methanolic solution of DPPH was added and allowed to stand for 20 min at 27°C. The absorbance of the sample was measured at 517 nm. Percentage radical scavenging activity of the sample was calculated as:

% DPPH radical scavenging activity = (Control OD-Sample OD / Control OD) × 100

1.3.2. Ferric Reducing Antioxidant Power Assay (FRAP)

The FRAP assay was used to estimate the reducing capacity of the sample [15]. The FRAP reagent contained 2.5 ml of a 10 mM TPTZ [2, 4, 6-tri (2-pyridyl)-1, 3, 5-triazine] solution in 40 mM HCl, 2.5 ml of 20 mM $FeCl_3$. $6H_2O$ and 25 ml of 300 mM acetate buffer (pH 3.6). It was freshly prepared and warmed at 37 °C. 900 µl FRAP reagent was mixed with 90 µl water and 10 µl of the extract of bamboo kernel flour (Ba-T, Ba-K, Ds-T and Ds-K). The reaction mixture was incubated at 37 °C for 30 minutes and the absorbance was measured at 593 nm.

1.3.3. Metal Chelating Activity

The chelating of ferrous ions by the extract of bamboo kernel flour (Ba-T, Ba-K, Ds-T and Ds-K) was estimated [16]. Briefly, 50 µl of 2 mM $FeCl_2$ was added to sample extracts. The reaction was initiated by the addition of 0.2 ml of 5 mM ferrozine solution. The mixture was vigorously shaken and left to stand at room temperature for 10 min. The absorbance of the solution was thereafter measured at 562 nm. The results were expressed as EDTA equivalent.

1.3.4. Hydroxyl Radical Scavenging Activity

The scavenging activity of the extract of bamboo kernel flour (Ba-T, Ba-K, Ds-T and Ds-K) on hydroxyl radical was measured according to the method of Klein [17]. Different concentrations of the extract (20 - 100 µg) were added with 1ml of iron-EDTA solution (0.13 % ferrous ammonium sulfate and 0.26 % EDTA), 0.5 ml of EDTA solution (0.018 %), and 1ml of Dimethyl sulfoxide (DMSO) (0.85 % v/v in 0.1 M phosphate buffer, pH 7.4). The reaction was initiated by adding 0.5 ml of ascorbic acid (0.22 %) and incubated at 80-90 °C for 15 min in a water bath. After incubation, the reaction was terminated by the addition of 1ml of ice-cold TCA (17.5 % w/v). Three milliliters of Nash reagent (75.0 g of ammonium acetate, 3 ml of glacial acetic acid and 2 ml of acetyl acetone were mixed and raised to 1 L with distilled water) was added and left at room temperature for 15 min. The intensity of the colour formed was measured spectroscopically at 412 nm against reagent blank. The hydroxyl radical scavenging activity (%) (HRSA) was calculated as

%HRSA (%) = (Control OD-Sample OD / Control OD) × 100

1.3.5. Nitric Oxide Radical Scavenging Activity

The nitric oxide radical scavenging activity of the extract of bamboo kernel flour (Ba-T, Ba-K, Ds-T and Ds-k) was measured according to the method of Sreejayan and Rao [18]. 3 ml of 10 mM sodium nitroprusside in 0.2 M phosphate buffered

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saline (pH 7.4) was mixed with different concentrations (20-100 µg) of solvent extracts and incubated at room temperature for 150 min. After incubation time, 0.5 ml of Griess reagent (1 % sulfanilamide, 0.1 % naphthylethylene diamine dihydrochloride in 2 % H_3PO_4) was added. The absorbance of the chromophore formed was read at 546 nm. Percentage of nitric oxide radical scavenging activity of the sample was calculated as

Nitric oxide radical scavenging activity (%) = (Control OD-Sample OD / Control OD) x 100

1.3.6. Superoxide Radical Scavenging Activity

Superoxide radicals were generated by a modified method of Beauchamp and Fridovich [19]. The assay was based on the capacity of the sample to inhibit farmazan formation by scavenging the superoxide radicals generated in riboflavin-light-NBT system. Each 3 ml reaction mixture contained 50 mM sodium phosphate buffer (pH 7.6), 20 mg riboflavin, 12 mM EDTA, 0.1 mg NBT and various concentrations (20 – 100 µg) of bamboo kernel flour (Ba-T, Ba-K, Ds-T and Ds-K) extracts. Reaction was started by illuminating the reaction mixture with sample extract for 90 seconds. Immediately after illumination the absorbance was measured at 590 nm. The entire reaction assembly was enclosed in a box lined with aluminium foil. Identical tubes with reaction mixture kept in dark served as blank. The percentage inhibition of superoxide anion generation was calculated as

Inhibition (%) = (Control OD-Sample OD / Control OD) x 100

1.4.Gas chromatography-Mass spectroscopy condition

The total of bamboo seed varieties of *Bambusa arundinacea* and *Dentrocalamus strictus* grown in Tamil Nadu and kerala was analyzed by GC/MS. Preparation of extract: 2 µl of the ethanolic extract of bamboo seed was employed for GC/MS analysis. Instruments and chromatographic conditions: GC-MS analysis was carried out on a GC Clarus 500 Perkin Elmer and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column Elite-5MS (5% Diphenyl /95% Dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 2µl was employed, injector temperature 250°.

The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C/min, then 5°C/min to 280°C/min, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 45 to 450 Da. The name, molecular weight and structure of the components of the test materials were ascertained [20].

3.RESULTS AND DISCUSSION

1.5.Gas chromatography-Mass spectroscopy

The present investigation was carried out to determine the components present in ethanolic extracts of bamboo seed varieties by GC-MS. The analysis gave the GC-MS Chromatogram which indicated a mixture of compounds with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) in the ethanol

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extract of bamboo seed variety are presented in Fig. 1.

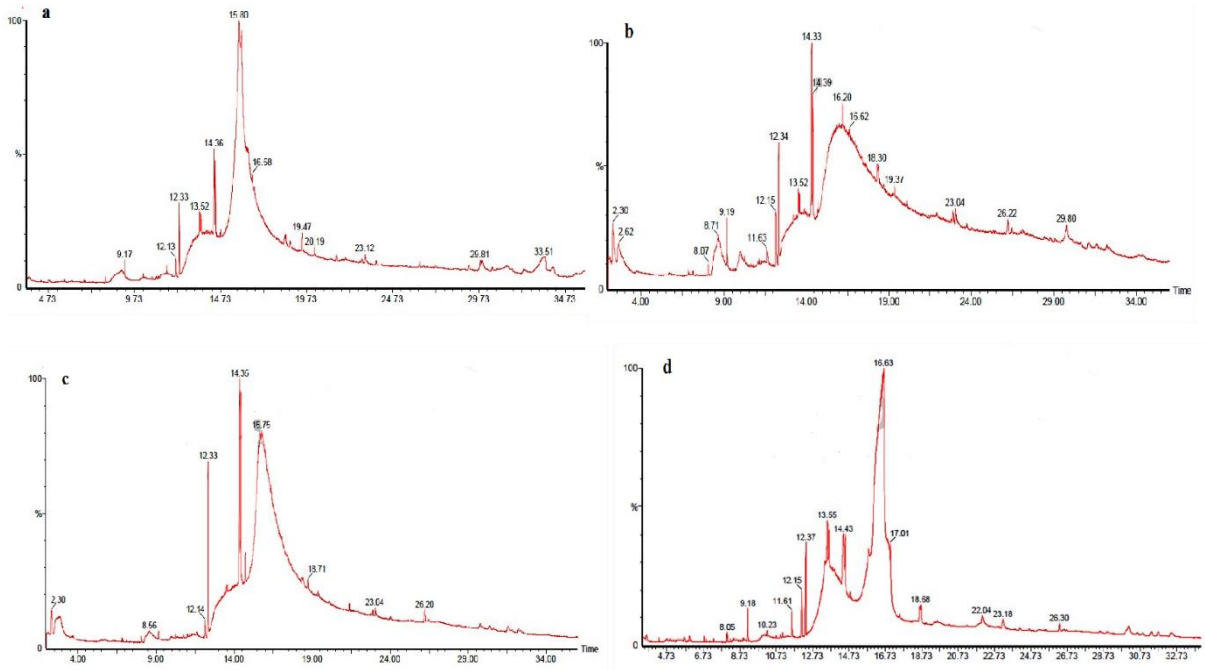

Fig. 1. (a-d): GC-MS chromatogram of Ba-T, Ba-K, Ds-T and Ds-K kernel flour

Table1. Activity of the compound

Name of the compound	Activity
Lactose	Preservative
à-D-Glucopyranoside,O-à-D-glucopyranosyl-(1.fwdarw.3)-á-D-fructofuranosyl	Preservative
Tetradecanoic acid, 12-methyl-, methyl ester	Antioxident, cancer preventive, cosmentic, hypocholesterolemic, lubricant and nematicide.
Hexadecanoic acid, ethyl ester	Antioxident, hypocholesterolemic, nematicide, pesticide, anti androgenicflavor, hemolytic and 5-alpha reductase inhibitor.
Linoleic acid ethyl ester	Anti-inflammatory, hypocholesterolemic, cancer preventive, hepato protective, nematicide, insectifuge, antihistaminic, antieczemic antiacne, 5-alpha reductase inhibitor, antiandrogenic,antiarthritic,anticoronary and insectifuge.
Octadecanoic acid	Antibacterial
9,12-Octadecadienoic acid (Z,Z)-	Antiinflammatory, hypocholesterolemic, cancer preventive, nematicide, insectifuge, antihistaminic, antieczemic, antiacne.
(E)-9-Octadecenoic acid ethyl ester	Raises VLDL and Lowers HDL Cholesterol

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n-Hexadecanoic acid	Antioxidant, Hypochloesterolemic, Nematicide, Pesticide, Lubricant, Antiandrogenic, Haemolytic, 5-Alpha reductase inhibitor
12-Methyl-E,E-2,13-octadecadien-1-ol	Antibacterial
Oleic Acid	Cancer preventive, Anemiagenic, Insectifuge, Antiandrogenic, Dermatitigenic.
[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	Hemolytic, pesticide, Skin irritant, hypocholesterolemic
7-Methyl-Z-tetradecen-1-ol acetate	Anti-cancer, antiinflammatory, hepatoprotective
Z-10-Tetradecen-1-ol acetate	Unsaturated alcoholic compound
Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	Antibacterial
2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde	No activity
Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy	No activity
Dodecane, 2,6,10-trimethyl-	Antioxidant, Antibacterial, COX-1
Hexadecane	No activity
Heptadecane	No activity
Squalene	Antioxidant, Antitumor
2H-Pyran, 2-(7-heptadecynyloxy)tetrahydro-	Antimicrobial
Cholestan-3-ol, 2-methylene-, (3á,5à)-	Antimicrobial Antiinflammatory Anticancer ,Diuretic Antiasthma Antiarthritic
á-Sitosterol	Antimicrobial, Anticancer, Antiarthritic, Antiasthma Diuretic, Anti-inflammatory

The peak of the chromatogram (75.82, 77.58, 86.56 and 71.86%), the major component was identified as 9,12-Octadecanoic acid (Z,Z) in *Bambusa arundinacea* grown in Tamil Nadu and kerala, *Dentrocalamus strictus* grown in Tamil Nadu and Kerala with molecular 280. Similar results were observed in leaves of *Cleome chelidonii (L.) Linn var. (CC)* Synonyms / other Latin name are Polanisia chelidonii DC, [family: Capparaceae] most places throughout the India and Tropical and warm temperate regions. It is grown as perennials throughout dry seasons and it contain the Compounds like 9,12,15-Octadecadienoic acid (Z,Z,Z)-, methyl ester (20.61%), 9,12-Octadecenoic acid (Z,Z)-, methyl ester (3.10%), n – decanoic acid (0.69%), Hexadecanoic acid and Squalene (0.55%). More than 35 compounds have been identified (Parimalakrishnan *et al*., 2015). Followed by Octadecanoic acid, n-

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Hexadecanoic acid, Linoleic acid ethyl ester,(E)-9-Octadecenoicacid ethyl ester, Hexadecanoic acid ethyl ester, 12-Methyl-E,E-2,13-octadecadien-1-ol and Tetradecanoic acid, 12-methyl-,ester and Oleic acid were present in bamboo seed varieties with different peak levels and molecular weight and these fatty acids are play the varies beneficial roles to human beings and it was explained in Table 1. The similar results were also observed in cereals of wheat, rye and barley also contain Linoleic acid, oleic acid and palmitic acid studied by Zhou *et al*.,(1996) [21] reported that the major fatty acids (FA) in spring and winter wheat cultivars shows the presence of linoleic, palmitic and oleic acids, whereas α-linolenic and stearic acids are minor components and According to Kanimozhi and Ratha Bai (2012) reported that this analysis revealed that ethanolic extract of *Coriandrum sativum* contains 9-Octadecenoic Acid (Z)- ethyl ester(56.68%), Linoleic Acid ethyl ester(13.64%), Ethyl Hexadecanoate(7.69%), Alpha.-Monoolein(6.66%) in a high percentage [22]. The most prevailing compound 9-Octadecenoic Acid (Z)- ethyl ester (ethyl oleate) used as a solvent for pharmaceutical drug reparation, it acts as a drug for intramuscular drug delivery, in some cases to prepare the daily doses of progesterone in support of pregnancy and Cristina Botinești*ean et al*., (2012) observed that the tomato seed oil by Gas Chromatography combined with Mass Spectrometry [23]. Tomato seed oil that was used for analysis has been obtained by cold pressed extraction method. It is known that individual fatty acids can be identified by GC because of their different retention times, the samples of tomato seeds oil were testifier to bring them into a vaporous phase, transforming the fatty acid from tomato seed oil into fatty acids methyl esters. The results showed that the major component of tomato seed oil was linoleic acid (48,2%), followed by palmitic acid (17.18%) and oleic acid (9.2%), all the fatty acids were expressed in methyl esters. It can be concluded that tomato seed oil is an excellent source of essential fatty acids omega-6 (linoleic acid) and omega-9 (oleic acid). The other component like sugars, Esters, Acetate, Alkanes and Aldehyde are also identified with different peaks levels in bamboo seed varieties and it was act as Antibacterial, Preservative and hypocholesterolemia effect. Lin *et al.*, (2010) stated that the most abundant aldehyde in indica and japonica rice varieties was hexanal and nonanal; ketone compound was 6-10,14 trimethyl 2 penta decanone [24]. Among identified phytochemicals, Dodecane, 2,6,10-trimethyl- and Squalene were possessing antioxidant Activity. Recently it has been found that Squalene possesses chemo-preventive activity against the colon carcinogenesis [25]. Cholestan-3-ol, 2-methylene-, (3á,5à)- and á-Sitosterol are antiarthritic and anticancer agents. The geographical location and agronomical characters are the major reason for present or absent of above-mentioned components within the variety. Bajwa et al.,(2021) studied major phenolic compounds identified in shoots are ferulic acid, p-coumaric acid, caffeic acid, protocatechuic acid, p-hydroxybenzoic acid, catechin, syringic acid, and chlorogenic acid while major sterols are β-sitosterol, campesterol, stigmasterol, cholesterol, ergosterol, and stigmastanol [26]. According to Upadhyay *et al*., (2010) studied the phytochemical analysis of *lawsonia inermis*. L leaf and

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revealed that color, number and leaf size showed morphological variation in the *Lawsonia inermis* populations; these are strongly influenced by environmental factors [27]. Morphological variation is apparently the result of an adaptive response to the environment; for example, variation in growth traits and phonological traits is associated with a latitudinal and altitudinal range or by contrasting climatic conditions. The observed trend of morphological variation made mention of adaptation to the contrasting micro-edaphic conditions prevailing for these groups and this was supported by the significant correlation with soil physicochemical characteristics. The greater discrimination power of adaptation micro edaphic conditions compared to the geographical regions of origin of accession in this study clearly indicated the greater importance of environmental factors (soil texture, soil chemical characteristics, and annual rainfall) than geographical location, in discriminating populations.

1.6.Antioxidant Profile

Antioxidants are the substances that inhibit oxidation. Cells in the human body may function poorly or die if oxidation occurs. To prevent free radical damage, body has a defense system of antioxidants. Antioxidants are the molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged [28]. Antioxidants can react with free radicals during the oxidation process by acting as a reactive species, scavenger and liberating catalysts, so antioxidants can be used to reduce the oxidative process but they are not 100 % effective [29]. Bamboo has been used over centuries by the humans both in daily life and for medicinal purpose in China and other Asian countries. The earliest scientific evidence for use of bamboo in traditional medicine dates back to 1963 [30]. This marked the beginning of the use of bamboo as medicine which was followed by series of research carried out by different workers [31–37]. *B. arundinacea* possess several characteristic identifying features in their pharmacognostical as well as physicochemical profiles. Phytochemical analysis exhibited vital information regarding the bio-constitutents present in the seeds which implies their possible therapeutic potential [38]. The bioactivities of some constituents present in the seeds can ensure a likely utilization of the seeds in the manufacturing of medicines in future. There was no significant investigation on antioxidant activity of bamboo paddy and its byproducts. Hence the antioxidant activity profile of dehusked bamboo kernel flour of two bamboo varieties was studied through DPPH radical scavenging activity, FT-IR assay, hydroxyl radical scavenging activity, nitric oxide radical scavenging activity, superoxide radical scavenging activity and metal chelating ability.

1.6.1. DPPH Radical Scavenging Activity

The DPPH free radical method has been used extensively to evaluate reducing substances, based on the reduction of ethanolic DPPH solution in the presence of a proton donating substance, resulting in the formation of diamagnetic molecules [39]. DPPH is commercially available nitrogen centered stable free radical which is destroyed by a free radical scavenger. The method is based on the measurement of the loss of deep purple colour of DPPH after reaction with the test compound functioning as a proton radical scavenger or hydrogen donor [40]. The DPPH radical

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scavenging activity of Ba-T, Ba-K, Ds-T and Ds-K kernel flour is depicted in Table 2 and Fig. 2 .

TABLE 2: ANTIOXIDANT AND RADICAL SCAVENGING ACTIVITY (IC_{50} VALUE) OF BAMBOO KERNEL FLOUR

Antioxidant Assay	Standards	Ba-T	Ba-K	Ds-T	Ds-K
DPPH IC_{50} (µg/ml) Std: Ascorbic acid	27.23±0.82*	46.04±0.06^{ax}*	79.62±1.83^{bx}*	55.45±2.39^{ay}*	52.57±0.04^{by}*
FRAP IC_{50} (µg/ml) Std: Ascorbic acid	39.11±0.18*	85.99±2.25^{ax}*	80.85±2.56^{ax}*	59.14±3.32^{ay}*	52.12±9.32^{ay}*
Hydroxyl radical scavenging IC_{50} (µg/ml) Std: Ascorbic acid	44.24±1.42*	56.16±0.00^{ax}*	52.02±0.04^{bx}*	64.08±0.00^{ay}*	59.72±0.61^{by}*
Nitric oxide radical scavenging activity(µg/ml) Std: Ascorbic acid	25.23±1.10*	25.15±4.60^{ax}	26.17±0.04^{ax}	35.48±0.00^{ay}*	31.77±0.00^{ay}*
Superoxide radical scavenging activity(µg/ml) Std: Ascorbic acid	34.16±0.09*	36.28±0.00^{ax}*	35.71±0.00^{bx}*	44.47±0.03^{ay}*	40.15±0.04^{by}*
Metal chelating ability (mg EDTA/g) Std: Ascorbic acid	45.23±0.01*	50.47±0.01^{ax}*	47.87±0.01^{bx}*	57.39±0.00^{ay}*	53.48±0.00^{by*}

Values are the average of three determinants. Different alphabets in superscript (a, b) indicates significant geographical difference between means at $p<0.05$ using LSD test; (x, y) indicates the significant varietal difference between the means at $p<0.05$ using LSD test; * indicates significant difference between the sample means and standard mean at $p<0.05$ using LSD test.

TABLE 3: DPPH RADICAL SCAVENGING ACTIVITY OF BAMBOO KERNEL FLOUR AT VARIOUS CONCENTRATIONS

Concentration(µg/ml)	Ba-T	Ba-K	Ds-T	Ds-K
20	22.52±0.04	23.09± 1.78	16.09±0.00	20.08±0.04

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40	42.45±1.41	42.45±1.41	29.11±0.08	34.15±0.04
60	72.21±7.05	72.19±7.03	55.18±18.50	70.24±0.04
80	78.78±0.70	78.78±0.70	70.35±0.04	73.36±0.04
100	89.80±5.88	89.80±5.88	79.50±0.04	82.52±0.00

Value indicates the percentage of inhibition of DPPH radical

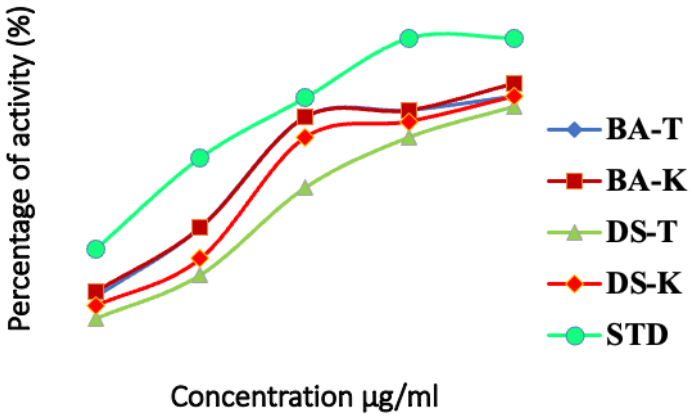

Fig. 2: DPPH activity in aqueous extract of Ba-T, Ba-K, Ds-T and Ds-K

DPPH radical scavenging activity was studied at 20-100 µg/ml concentration (Table 3 and Fig. 2) and the different concentration of aqueous extract of Ba-T (Y=0.814×+12.54), (Y=0.814×+12.49) and (Y=0.813×+12.50); Ba-K (Y=0.798×+13.84), (Y=0.798×+13.72) and (Y=0.799×+13.76); Ds-T (Y=0.840×+2.291), (Y=0.839×+2.261), and (Y=0.841×+5.673); Ds-K (Y=0.820 x+6.909), (Y=0.820 x+6.841) and (Y=0.821 x+6.769) (Fig. 2) was linearly correlated with superoxide radical scavenging activity. According to IC_{50} value of DPPH activity (Table 3), the significantly highest activity was ($p<0.05$) found in Ba-T and Ba-K when compared to Ds-T and Ds-K. Moko *et al* (2014) stated that the lower IC_{50} value indicated the strong capability of samples to catch free radical of DPPH [41]. The scavenging activity of bamboo kernel flour was significantly low when compared to standard ascorbic acid. Biswas *et al* (2011) observed the IC_{50} value in *Jaladhi 1* rice variety was at the range of 23.23 µg/ml that exhibit higher DPPH radical scavenging activity which was comparable to the present study of bamboo seed varieties [42]. The variation in the DPPH radical scavenging activity among bamboo seed varieties could be attributed to the variation in the composition and secondary metabolites of crops based on the genetic diversity and variations [43,44]. Different extracts of bamboo leaves proved to possess radical scavenging, metal chelating, ferric reducing and nitric oxide scavenging capability. The present study has verified the usefulness of bamboo leaves as effective antioxidant and a rich source of phenol and flavonoids [45].

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Pujiarti *et al*., (2020) studied that higher antioxidant activity was shown by the 70% ethanol-soluble extract of *G. verticillata*, followed by D. asper, and the G. verticillata leaf extract exhibited effective DPPH radical-scavenging activity [46]. It contained high amounts of TPC and TFC.

1.6.2. Ferric Reducing Antioxidant Power (FRAP) Assay

The ferric reducing antioxidant power (FRAP) assay measures the reduction of ferric iron ($Fe^{3+)}$ in the presence of antioxidants, which are reductants with half-reaction reduction potentials above Fe^{3+}/Fe^{2+} [47]. The FRAP activity of bamboo kernel flour was increased with concentration (Table 4 and Fig 3). The different concentration of aqueous extract of Ba-T (Y=0.521×+4.668), (Y=0.510×+5.064) and (Y=0.556×+3.916); Ba-K (Y=0.538×+4.866), (Y=0.63×+1.128) and (Y=0.589×+2.215); Ds-T (Y=0.743×+3.714), (Y=0.725×+6.061) and (Y=0.712×+11.16); Ds-K (Y=0.729x+6.239), (Y=0.643x+15.22) and (Y=0.554x+26.54) (Fig 3). The FRAP of Ba-T and Ba-K was significantly ($p<0.05$) low when compared to Ds-T and Ds-K kernel flour. The difference between varieties may be the difference in phenolic contents (polyphenolics and anthocyanin extracts) and/or electron-donating activity [48]. As per the IC_{50} value, the Ba-T and Ba-K showed high reducing capacity (50 %) of ferric ion at lowest concentration and it was significant at $p<0.05$. Table 4 explained that highest level of phenolics was observed in *Bambusa arundanacea* variety than *Dentrocalamus strictus* variety. The FRAP of bamboo kernel flour was significantly lower than the content ascorbic acid. Tundis *et al*., (2023) studied that bamboo leaves of phyllostacys edulis J.Houz has anti-inflammatory and antioxidant properties of BL and BS, corroborating their different potential applications in the nutraceutical, cosmetic and pharmaceutical industries [49].

TABLE 4 : FERRIC REDUCING ANTIOXIDANT POWER OF BAMBOO KERNEL FLOUR AT VARIOUS CONCENTRATION

Concentration (µg/ml)	Ba-T	Ba-K	Ds-T	Ds-K
20	13.16±1.44	14.48±0.45	18.21±0.01	25.96±7.06
40	23.11±2.24	27.25±4.22	35.15±6.22	39.16±5.74
60	26.12±0.03	41.80±4.71	58.52±4.06	63.21±3.50
80	43.58±1.94	60.49±4.59	65.23±0.16	69.15±1.37
100	57.9±4.03	72.17±2.91	75.86±1.78	75.19±2.85

Value indicate the percentage of ferric reducing power

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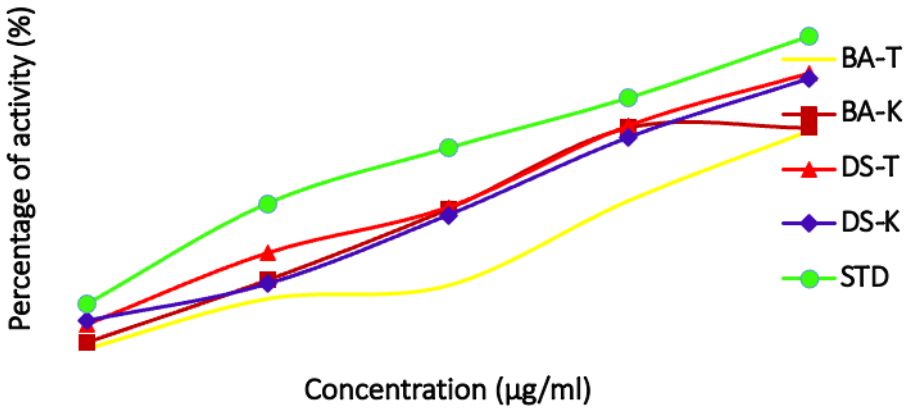

Fig. 3: FRAP activity in aqueous extract of Ba-T, Ba-K, Ds-T and Ds-K

1.6.3. Hydroxyl Radical Scavenging Activity

Hydroxyl radical is the most reactive free radical in the biological system and it has been regarded as the highly damaging to almost every molecule found in the biological system. It can conjugate with nucleotides in DNA and cause strand breakage which leads to ultimately mutagenesis, carcinogenesis and cytotoxicity [50]. The hydroxyl radical scavenging activity of bamboo kernel flour was gradually increased with increased concentration and the studied varieties of bamboo kernel flour were significantly ($p<0.05$) less effective than ascorbic acid in destroying the hydroxyl radicals (Table 5) and (Fig 4). The different concentration of aqueous extract of Ba-T (Y=0.599×+16.36), (Y=0.599×+16.36) and (Y=0.599×+16.36); Ba-K (Y=0.604×+18.56), (Y=0.604×+18.56) and (Y=0.605×+18.55); Ds-T (Y=0.610×+10.91), (Y=0.610×+10.91) and (Y=0.610×+10.910); Ds-K (Y=0.610x+13.78), (Y=0.610x+13.78) and (Y=0.610x+13.13) (Fig. 4). According to the IC_{50} value, the Ba-K showed highest inhibition activity at lowest concentration followed by Ba-T, Ds-K and Ds-T (Table 4.15). The IC_{50} value of non-germinated rice variety of superjami (48.57±0.71) reported by Im Chung (2016) was comparable to IC_{50} value of Ba-K and Ba-T. Colombo *et al.*, (2024) studied that rice (*Oryza sativa* L.) production and consumption is increasing worldwide and many efforts to decrease the substantial impact of its byproducts are needed. In fact, rice byproducts are rich in secondary metabolites (phenolic compounds, flavonoids, and tocopherols) with different types of bioactivity, mainly antioxidant, antimicrobial, antidiabetic, and anti-inflammatory, which make them useful as functional ingredients [51].

TABLE 5: HYDROXYL RADICAL SCAVENGING ACTIVITY OF BAMBOO KERNEL FLOUR AT VARIOUS CONCENTRATION

Concentration (µg/ml)	Ba-T	Ba-K	Ds-T	Ds-K

[DOI: 10.22034/FSCT.22.160.13]

20	31.16±0.00	33.25±0.00	26.11±0.00	29.18±0.00
40	39.27±0.00	42.36±0.00	34.22±0.00	37.26±0.04
60	50.34±0.00	52.45±0.00	45.32±0.00	47.40±0.00
80	60.44±0.00	62.57±0.00	55.43±0.00	58.51±0.00
100	80.55±0.00	83.64±0.00	76.54±0.00	79.60±0.00

Value indicates the percentage of hydroxyl radical scavenging ability

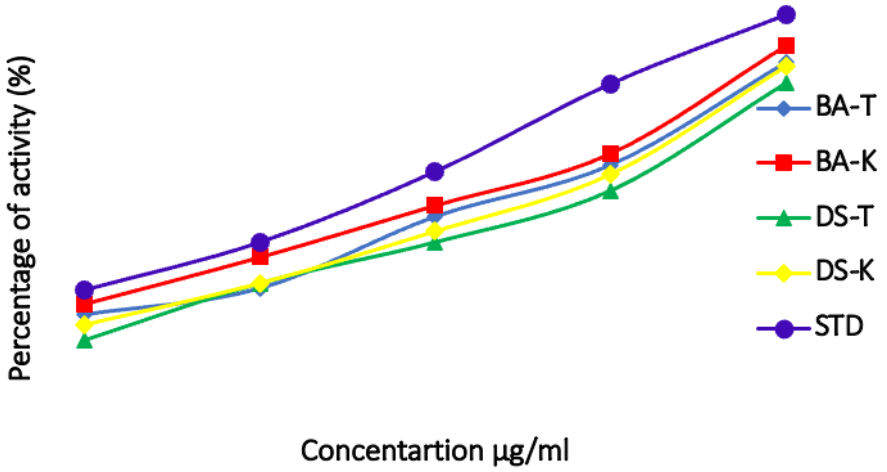

Fig.4: Hydroxyl radical scavenging activity in aqueous extract of Ba-T, Ba-K, Ds-T and Ds-K

1.6.4. Nitric Oxide Radical Scavenging Activity

Nitric oxide has also been involved in a variety of biological functions including neurotransmission, vascular homeostatic, antimicrobial and antitumor activities [18,52–57].

The nitric oxide radical scavenging activity was performed by comparing with ascorbic acid standard. The nitric acid radical scavenging activity was high in Ba-K (94.76±0.04) at 100 µl concentration followed by Ba-T, Ds-K and Ds-T (Table 6 and Fig. 5). The different concentration of aqueous extract of Ba-T (Y=0.714×+29.16), (Y=0.714×+29.15) and (Y=0.720×+31.17); Ba-K (Y=0.720×+31.17), (Y=0.720×+31.17) and (Y=0.720×+31.17); Ds-T (Y=0.720×+24.45), (Y=0.720×+24.45) and (Y=0.719×+24.48); Ds-K (Y=0.700X+27.76), (Y=0.700X+27.76) and (Y=0.7X+27.75) (Fig. 4). The IC_{50} value of bamboo kernel flour represented in Table 6 and reveals that *Bambusa arundanacea* variety exhibited strongest nitric oxide radical scavenging activity and equal to ascorbic acid standard, while *Dentrocalamus strictus* variety exhibited significantly low inhibition activity than Ba-K, Ba-T.

TABLE 6: NITRIC OXIDE RADICAL SCAVENGING ACTIVITY OF BAMBOO KERNEL FLOUR AT VARIOUS CONCENTRATION

Concentration (µg/ml)	Ba-T	Ba-K	Ds-T	Ds-K

[DOI: 10.22034/FSCT.22.160.13]

20	32.25±0.00	34.37±0.00	27.06±0.00	30.16±0.00
40	64.37±0.00	67.44±0.06	61.16±0.00	63.26±0.04
60	83.44±0.00	84.56±0.04	78.27±0.00	80.35±0.00
80	88.54±0.00	90.70±0.00	84.38±0.00	86.45±0.00
100	91.65±0.00	94.76±0.04	87.46±0.04	88.56±0.00

Value indicates the percentage of nitric oxide radical scavenging ability

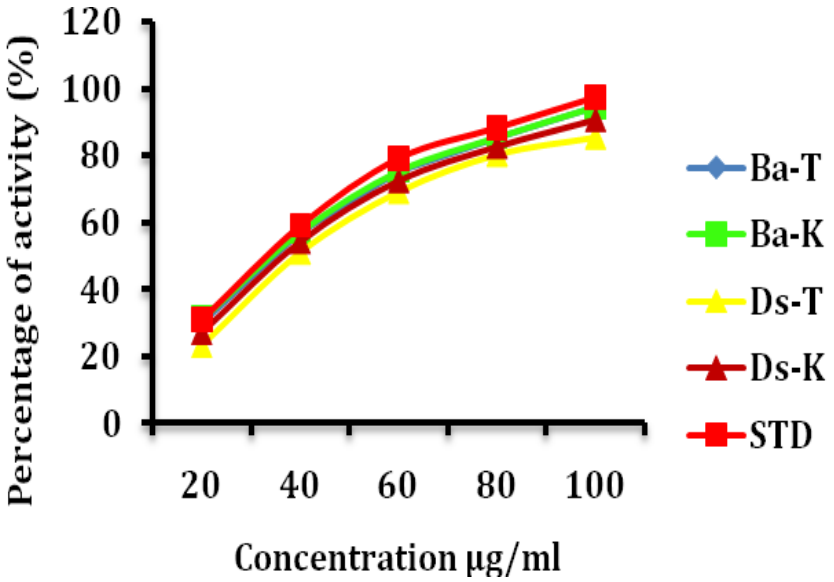

Fig. 5: Nitric oxide radical scavenging activity in aqueous extract of Ba-T, Ba-K, Ds-T and Ds-K

1.6.5. Superoxide Radical Scavenging Activity

The SOD-like activity measures the ability of the extract to catalyze the conversion of superoxide radicals into hydrogen peroxides, providing a defence mechanism against oxidative damage [58]. The superoxide radical scavenging activity of was increased significantly ($p<0.05$) (Table 7 and Fig. 6) as concentration of the extract increased.

TABLE 7: SUPEROXIDE RADICAL SCAVENGINGACTIVITY OF BAMBOO KERNEL FLOUR AT VARIOUS CONCENTRATION

Concentration (µg/ml)	Ba-T	Ba-K	Ds-T	Ds-K
20	30.23±0.00	31.23±0.00	23.36±0.04	27.18±0.00
40	57.36±7.11	57.35±0.06	51.15±0.00	54.26±0.04
60	75.47±0.00	75.47±0.04	69.27±0.00	72.42±0.00
80	85.58±0.00	85.58±0.00	80.36±0.04	82.55±0.00
100	94.70±0.00	94.70±0.04	85.50±0.04	90.59±0.04

[DOI: 10.22034/FSCT.22.160.13]

Value indicates the percentage of superoxide radical scavenging ability

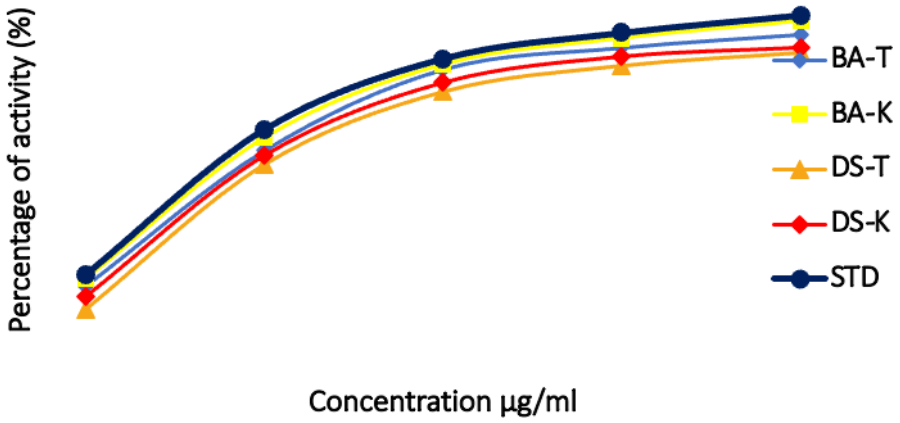

Fig. 6: Superoxide radicalscavenging activity in aqueous extract of Ba-T, Ba-k, Ds-T and Ds-K

The different concentration of aqueous extract of Ba-T (Y=0.785×+21.52), (Y=0.785×+21.52) and (Y=0.785×+21.52); Ba-K (Y=0.775×+22.32), (Y=0.775×+22.32) and (Y=0.775×+22.32);Ds-T (Y=0.767×+15.91), (Y=0.767×+15.91) and (Y=0.768×+15.83): Ds-K (Y=0.775x+18.86), (Y=0.775x+18.86) and (Y=0.775x+18.89) (Fig. 6) was linearly correlated with superoxide radical scavenging activity. The superoxide radical scavenging activity *Dentrocalamus strictus* variety was significantly ($p<0.05$) greater than *Bambusa arundinacea* variety. The superoxide radical scavenging activity of bamboo kernel flour was significantly ($p<0.05$) less than the ascorbic acid standard.

1.6.6. Metal Chelating Ability

Ferrozine can quantitatively form complexes with Fe^{2+} in the presence of chelating agents; the complex formation is disrupted resulting to decrease in red colour of the complex. Measurements of colour reduction make possible estimation of the metal chelating activity [59]. The result in Table 8 and Fig 7 f showed the interaction of bamboo kernel flour with iron. The different concentration of aqueous extract of Ba-T (Y=0.730×+13.17), (Y=0.730×+13.17) and (Y=0.730×+13.17); Ba-K (Y=0.704×+16.30), (Y=0.704×+16.30) and (Y=0.703×+16.33); Ds-T (Y=0.715×+8.956), (Y=0.715×+8.956) and (Y=0.715×+8.955); Ds-K (Y=0.723×+11.33), (Y=0.723×+11.33) and (Y=0.723×+11.33) was linearly correlated with metal ion chelating activity. The chelating ability of bamboo kernal flour was significantly ($p<0.05$) less than the ascorbic acid standard. The Ba-K (47.87±0.01 µg/ml) was found that significantly higher chelating activity with lowest concentration (Table 8).

TABLE 8: METAL CHELATING ACTIVITY OF BAMBOO KERNAL FLOUR AT VARIOUS CONCENTRATION

[DOI: 10.22034/FSCT.22.160.13]

Concentration (µg/ml)	Ba-T	Ba-K	Ds-T	Ds-K
20	30.18±0.00	32.26±0.00	25.06±0.00	27.15±0.00
40	39.28±0.00	42.37±0.00	35.17±0.00	38.26±0.04
60	55.36±0.04	57.48±0.00	50.28±0.00	53.37±0.00
80	74.50±0.00	73.58±0.00	69.36±0.04	72.48±0.00
100	85.61±6.43	87.06±0.04	79.51±0.00	82.37±0.00

Value indicates the percentage of metal chelating ability

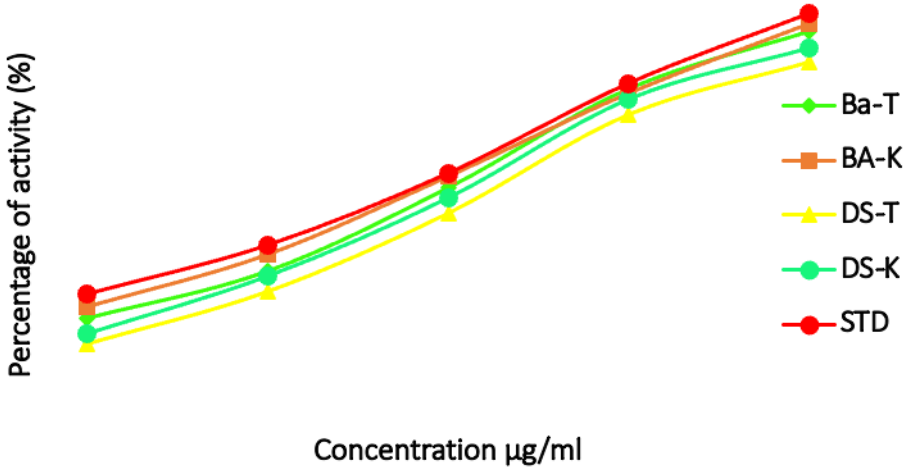

Fig. 7: Metal chelating ability in aqueous extract of Ba-T, Ba-K, Ds-T and Ds-K

CONCLUSION

In the current study, 24 compounds were identified in the ethanol extract of bamboo seed varieties through Gas Chromatography-Mass Spectrometry (GC-MS analysis). This analysis marks the initial step in comprehending the active principles present in these seeds and sets the stage for more comprehensive future studies. Further exploration into their pharmacological significance and diversity may expand our understanding of traditional medicinal practices. The DPPH activity's IC50 value revealed that Ba-T and Ba-K exhibited significantly higher activity ($p<0.05$) compared to Ds-T and Ds-K, while the scavenging activity of bamboo kernel flour was notably lower than that of the standard ascorbic acid. The FRAP values of Ba-T and Ba-K were significantly lower ($p<0.05$) than Ds-T and Ds-K kernel flour, possibly due to the higher phenolic levels in *Bambusa arundinacea* variety compared to *Dentrocalamus strictus* variety. In terms of the IC50 value for hydroxyl radical scavenging activity, Ba-K demonstrated the highest inhibition activity at the lowest concentration, followed by Ba-T, Ds-K, and Ds-T. Regarding the IC50 value for Nitric oxide radical scavenging activity, bamboo kernel flour of *Bambusa arundinacea* variety exhibited the strongest nitric oxide radical scavenging activity equivalent to the ascorbic acid standard, whereas *Dentrocalamus strictus* variety showed significantly lower inhibition activity than Ba-K, Ba-T, and ascorbic acid.

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The superoxide radical scavenging activity of *Dentrocalamus strictus* variety was notably higher ($p<0.05$) than that of *Bambusa arundinacea* variety, while the superoxide radical scavenging activity of bamboo kernel flour was markedly lower ($p<0.05$) than the ascorbic acid standard. The chelating ability of bamboo kernel flour was significantly lower ($p<0.05$) than that of ascorbic acid standard, with Ba-K displaying the highest activity.

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مطالعه فعالیت آنتی اکسیدانی و پروفایل GC-MS واریته های مختلف دانه بامبو

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چکیده

تحقیقات گسترده ای در مورد خواص آنتی اکسیدانی عصاره دانه بامبو، به ویژه از گیاه بامبو انجام شده است. دانه‌های بامبو به دلیل محتوای آنتی‌اکسیدانی بالایی که دارند، برای مزایای سلامتی بالقوه‌شان در مبارزه با بیماری‌های مزمن مرتبط با افزایش سن مانند دیابت، سرطان، آلزایمر، پارکینسون و بیماری‌های قلبی عروقی مورد توجه قرار گرفته‌اند. مصرف محصولات مشتق شده از بامبو به صورت روزانه ممکن است در کاهش خطر ابتلا به این شرایط نقش داشته باشد. آنتی اکسیدان ها نقش مهمی در صنایع غذایی و دارویی ایفا می کنند، زیرا به مقابله با رادیکال های آزاد کمک می کنند که می توانند محصولات را در طول پردازش و ذخیره سازی تجزیه کنند. اجزای شیمیایی عصاره اتانولی دانه بامبو با استفاده از کروماتوگرافی گازی-طیف‌سنجی جرمی با شناسایی بیست و چهار ترکیب مورد تجزیه و تحلیل قرار گرفت. اینها شامل ترکیباتی مانند ۹،۱۲-اوکتادکادینوییک اسید (Z,Z)-، اتیل استر اسید هگزادکانوئیک و -۱۲-متیل متیل استر تترادکانوییک اسید بود که در *Bambusa arundinacea* و *Dentrocalamus strictus* یافت شد. علاوه بر این، اتیل استر اسید لینولئیک و اسید اکتادکانوئیک در *B. arundinacea* غالب بودند، در حالی که اتیل استر -9-(E)اوکتادکانوئیک اسید، n-هگزادکانوئیک اسید، اولئیک اسید، و ۱۲-E-متیل، -2،13-E اوکتادکا دی ان بودند. -ol فقط در *Dentrocalamus strictus* یافت شد. سایر مواد مغذی گیاهی نیز در انواع دانه های بامبو وجود داشت که نشان دهنده استفاده بالقوه آنها در درمان بیماری های مختلف به عنوان آنتی اکسیدان و به عنوان یک جایگزین مناسب برای سایر غلات خصوصا برنج است.

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