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Research Article

PHYTOCHEMICAL SCREENING AND *IN VITRO* ANTIOXIDANT ACTIVITIES OF LEAF EXTRACTS OF *ALTERNANTHERA BRASILIANA* (L). KUNTZE AND *ALTERNANTHERA BETTZICKIANA* REGEL.

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ABSTRACT

Objectives: The present study was carried out to determine the phytochemical constituents and *in vitro* antioxidant activities of leaf extracts of *Alternanthera brasiliana* (L). Kuntze (*A. brasiliana)* and *Alternanthera bettzickiana* regel (*A. bettzickiana*).

Methods: Preliminary phytochemical screening was performed in petroleum ether, chloroform, acetone, ethanol, hydroethanolic and water extracts of leaves of *A. brasiliana* and *A. bettzickiana*. The level of alkaloids, flavonoids, total phenolic content, tannins, Vitamin C, Vitamin E, GSH, and total proteins were determined in hydroethanolic, ethanol, and water extracts. Antioxidant activity of the hydroethanolic leaf extracts of *A. brasiliana* and *A. bettzickiana* were determined by 2,2-diphenyl-1-picryl-hydrazyl-hydrate free radical scavenging assay, nitric oxide scavenging assay, superoxide anion scavenging assay, ferric reducing antioxidant power assay, total antioxidant capacity, and reducing power assay.

Results: The phytochemical screening of six different extracts of *A. brasiliana* and *A. bettzickiana* revealed the presence of various phytonutrients. Quantitative analysis of secondary metabolites in ethanol, hydroethanolic and water extracts of leaves of *A. brasiliana* and *A. bettzickiana* showed the presence of high amount of secondary metabolites in the hydroethanolic extract. *In vitro,* antioxidant assay of two plant extracts revealed that *A. bettzickiana* was more potent than *A. brasiliana* in scavenging free radicals.

Conclusion: The different extracts from *A. brasiliana* and *A. bettzickiana* and specifically the hydroethanolic extract of *A. bettzickiana* revealed several properties such as rich source of phytonutrients, higher free radical scavenging properties, and significant antioxidant capabilities. Therefore, the bioactive compound should be isolated in future studies and could be used as a safe and serve as a potential source of natural antioxidants.

Keywords: *Alternanthera brasiliana, Alternanthera bettzickiana*, Phytochemicals, Free radicals, Antioxidants.

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INTRODUCTION

Oxidative stress is the major causative factor in the induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, aging, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases, and others [1]. Free radicals are chemical species that contains one or more unpaired electrons. They are highly unstable and cause damage to other molecules by extracting electrons to attain stability [2]. Antioxidants are compounds that reduce oxidative stress in cells by neutralizing or scavenging reactive species by hydrogen donation [3]. Medicinal plants and their derivatives contain secondary metabolites which are potential sources of therapeutically effective medicines. Plant-based products are healthier, safer, and more reliable than synthetic products [4]. Commercially available synthetic antioxidants such as butylated hydroxyanisole and butylated hydroxytoluene are reported to be toxic to animals and human beings [5]. An alternative is the consumption of natural antioxidants from various food supplements and traditional medicines. Natural antioxidants which are ubiquitous in fruits, leaves, and flowers have been studied extensively as they are effective free radical scavengers and are presumed to be less toxic than synthetic antioxidants [6].

Alternanthera brasiliana and *Alternanthera bettzickiana* belonging to the family Amaranthaceae. This genus consists of approximately 80 species and is widespread genus with cosmopolitan distribution [7]. *A. brasiliana* is a herbaceous plant native to different countries such as Brazil, Australia, and India. It is commonly known in Brazil as Penicillin, Brazilian Joy Weed, grows easily on poor, and deforested soil [8]. *A. bettzickiana* Regel. is a species of flowering plant, known as red calico plant. The plant is used as an edible vegetable in Southeast Asia [9]. The present study was undertaken to assess the phytochemical constituents and antioxidant capabilities of edible herbs, *A. brasiliana and A. bettzickiana.*

METHODS

Chemicals

All the chemicals used in this study were purchased from HIMEDIA Pvt. Ltd., Bombay. The chemicals used were of analytical grade.

Collection of plant materials

Fresh leaves of *A. brasiliana* and *A. bettzickiana* were collected from SKM Siddha and Ayurveda, Erode. The plant specimens were identified and authenticated by Dr. G.V.S. Murthy, Botanical Survey of India, Coimbatore, with voucher number BSI/SRC/5/23/2015/Tech/100 for *A. brasiliana* (L). Kuntze - AMARANTHACEAE and BSI/SRC/5/23/2015/Tech/101 for *A. bettzickiana* (Regel) Voss - AMARANTHACEAE. The leaves of *A. brasiliana* and *A. bettzickiana* were separately shade dried and were coarsely powdered using a mechanical grinder.

Extraction

The shade dried coarsely powdered leaf samples of 250 g *A. brasiliana* and *A. bettzickiana* were extracted with solvents of increasing polarity such as petroleum ether, chloroform, acetone, ethanol, hydroethanolic, and water using hot continuous percolation process (Soxhlet) and the different extracts were concentrated using rotary vacuum evaporator (Buchi) at 50°C, dried in a vacuum desiccator and stored at −20°C till further use.

Preliminary phytochemical screening

The solvent extracts of leaves of *A. brasiliana* and *A. bettzickiana* were subjected to qualitative analysis to detect the presence of

various phytoconstituents. They were screened for the presence of phytonutrients such as alkaloids, flavonoids, carbohydrates, amino acids and proteins, sterols and triterpenoids, tannins and phenolics, quinones, and saponins. The preliminary phytochemical analysis was performed using standard methods [10,11].

Quantitative phytochemical analysis

Hydroethanolic, ethanol, and water extracts of leaves of *A. brasiliana* and *A. bettzickiana* were analyzed for quantitative estimation of alkaloids [12], flavonoids [13], total phenolics [14], tannins [15], and Vitamin C [16].

***In vitro* antioxidant assays**

The hydroethanolic leaf extracts of *A. brasiliana and A. bettzickiana* were subjected to 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay [17], nitric oxide (NO) scavenging assay [18], superoxide anion (SO) scavenging assay [19], ferric reducing antioxidant power (FRAP) assay [20], total antioxidant capacity (TAC) [21], and reducing power assay [22].

Statistical analysis

All the experiments were done in triplicates, and the results are expressed as mean±standard deviation.

RESULTS AND DISCUSSION

Phytochemical screening

Phytochemicals are bioactive, non-nutrient, naturally occurring compounds present in plants [23]. They usually occur as complex mixtures that differ among plant organs and stages of development [24]. The different solvent extracts of leaves of *A. brasiliana* and *A. bettzickiana* were evaluated for identification of efficient solvent system. The extracts were subjected to preliminary qualitative phytochemical investigations to determine phytoorganic constituents such as alkaloids, flavonoids, carbohydrates, amino acids and proteins, glycosides, sterols and triterpenoids, tannins and phenolics, quinones, and saponins. The results obtained are furnished in Table 1 for *A. brasiliana* and in Table 2 for *A. bettzickiana.*

Studies on the screening of the phytochemicals present in the tested six different extracts of *A. brasiliana* leaves revealed the presence of alkaloids, flavonoids, carbohydrates, amino acids and proteins, glycosides, sterols and triterpenoids, tannins and phenolics, and saponins in ethanol, hydroethanolic, and water extracts. Carbohydrates, sterols and triterpenoids, tannins and phenolics, and quinones were present in acetone extract. Petroleum ether and chloroform extracts showed the presence of sterols and triterpenoids, tannins, and phenolics.

Qualitative phytochemical screening of six different extracts of *A. bettzickiana* leaves revealed the presence of alkaloids, flavonoids, carbohydrates, amino acids and proteins, glycosides, sterols and triterpenoids, tannins and phenolic, quinones, and saponins in hydroethanolic and water extract. Ethanol extract contained all the phytonutrients present in hydroethanolic and water extract except sterols and triterpenoids and saponins. Acetone extract showed the presence of alkaloids, flavonoids, carbohydrates, amino acids and proteins, sterols and triterpenoids, and quinones. Alkaloids, carbohydrates, amino acids, and proteins were present in chloroform extract. Petroleum ether extract showed the presence of carbohydrates, amino acids and proteins, sterols and triterpenoids, tannins, and phenolics. Among the various solvent extracts studied in the leaves of *A. brasiliana* and *A. bettzickiana*, hydroethanolic followed by ethanol and water extracts were found to contain a maximum number of phytoconstituents. The phytochemical compounds detected in these plants were known to have medicinal importance. Tannins and flavonoids have been reported to possess biological activities that lead to prevention and management of many ailments [25]. Phenolics and flavonoids have been reported to be potent free radical scavengers [26]. Saponins have been found to have antimicrobial, anti-inflammatory, and hemolytic effects [27]. Terpenoids are known to be cytotoxic to tumor cells [28]. The presence of alkaloids, flavonoids, terpenoids, tannins, and phenolics in the leaf extracts of *A. brasiliana* and *A. bettzickiana* may preliminarily indicate their bioactivity, especially antitumor and antioxidant activity.

Quantitative phytochemical analysis

Medicinal plants are the rich source of alkaloids, phenolic acids, tannins, flavonoids, terpenoids, and other metabolites that act as primary antioxidants or free radical scavengers [29]. The quantitative phytochemical content of hydroethanolic, ethanol and water extracts of leaves *A. brasiliana* and *A. bettzickiana* were presented in Table 3.

Quantitative phytochemical analysis revealed that the hydroethanolic leaf extracts of *A. brasiliana* and *A. bettzickiana* retained a high amount of alkaloids, flavonoids, total phenolics, tannins, Vitamin C, Vitamin E, GSH, and total proteins. The ethanol and water extracts of *A. brasiliana* and *A. bettzickiana* leaves showed a lower amount of phytonutrients when compared to hydroethanolic extracts.

Alkaloids are the most efficient bioactive substances in plants [30]. Flavonoids and phenols are known to possess antioxidant activities. The presence of hydroxyl groups in their structures is responsible for antioxidant activity and their contribution to defense system against oxidative damage [31]. Antioxidants have the potential to exert their biological activity and scavenge reactive oxygen species (ROS) by quenching chain initiating catalysis, metal ion chelation, antioxidants, or by gene expression regulation [32]. Tannins are phenolic compounds, and plant phenolics are a major group of compounds that act as antioxidants or free radical scavengers [33]. Vitamin C is a powerful antioxidant because it can donate a hydrogen atom and form a relatively stable ascorbyl free radical. As a scavenger of ROS, ascorbate is shown to be effective against superoxide radical anion, hydrogen peroxide, hydroxyl radical, and singlet oxygen [34,35]. The non-enzymic antioxidant Vitamin E functions as peroxyl radical scavenger that terminates chain reactions [36]. GSH is a good scavenger of many free radicals such as O^{2-}, OH, and various lipid hydroperoxides and may help to detoxify many inhaled oxidizing air pollutants such as ozone, NO_2, and free radicals.

The results obtained from the quantitative phytochemical analysis of hydroethanolic, ethanol and water extracts of *A. brasiliana* and *A. bettzickiana* revealed the presence high amount of phytochemicals in hydroethanolic extract followed by ethanol and water extracts. Therefore, they hydroethanolic extract of both plant materials was selected for the evaluation of free radical scavenging and *in vitro* antioxidant activity.

Free radical scavenging potential and antioxidant properties

DPPH assay

The DPPH$^{\bullet}$ is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant compounds [37]. DPPH$^{\bullet}$ accepts an electron/hydrogen radical to become a stable diamagnetic molecule. Antioxidants on interaction with DPPH$^{\bullet}$ transfer electron/hydrogen atom to DPPH$^{\bullet}$ [38]. A freshly prepared DPPH$^{\bullet}$ solution is of deep purple color with an absorption maximum at 517 nm. In the presence of an antioxidant, this color disappears due to quenching of DPPH$^{\bullet}$ free radicals and converting them into a colorless product 2,2-dipenyl-1-picryl hydrazine [39]. In Table 4, DPPH radical scavenging activity of standard drug ascorbic acid and hydroethanolic leaf extracts of *A. brasiliana* and *A. bettzickiana* were shown.

At the minimum concentration of extracts used in this study (i.e., 50 µg/ ml), the percentage inhibition caused by *A. brasiliana* and *A. bettzickiana* had activity values corresponding to 31±0.01 and 35±0.02, respectively. Whereas at the highest concentration (i.e., 350 µg/ml), the activity values of *A. brasiliana* and *A. bettzickiana* were 66±0.01 and 73±0.01, respectively. It was observed that *A. bettzickiana* extract had the highest activity when compared to *A. brasiliana*. There was a

Table 1: Qualitative phytochemical analysis of various solvent extracts of *A. brasiliana* leaves

Phytochemical compound	Petroleum ether	Chloroform	Acetone	Ethanol	Hydroethanolic	Water
Alkaloids						
Dragendorff's	−	−	−	+	+	+
Wagner's	−	−	−	+	+	+
Flavonoids						
Alkaline reagent	−	−	−	+	+	+
Zinc test	−	−	−	+	+	+
Carbohydrates						
Molisch test	−	−	+	+	+	+
Fehling's test	−	−	+	+	+	+
Benedicts test	−	−	+	+	+	+
Amino acids and proteins						
biuret test	−	−	−	+	+	+
Millon's test	−	−	−	+	+	+
Ninhydrin test	−	−	−	+	+	+
Glycosides	−	−	−	+	+	+
Sterols and triterpenoids						
Liebermann-Burchard test	+	+	+	+	−	−
Salkowski's test	+	+	+	+	+	+
Tannins and phenolics						
Iodine test	+	+	+	+	+	+
Nitric acid test	+	+	+	+	+	+
Quininoes	−	−	+	+	+	−
Saponins	−	−	−	+	+	+

+: Positive, −: Negative. *A. brasiliana: Alternanthera brasiliana*

Table 2: Qualitative phytochemical analysis of various solvent extracts of *A. bettzickiana* leaves

Phytochemical compound	Petroleum ether	Chloroform	Acetone	Ethanol	Hydroethanolic	Water
Alkaloids						
Dragendorff's	−	−	−	+	+	+
Wagner's	−	+	+	+	+	+
Flavonoids						
Alkaline reagent	−	−	+	+	+	+
Zinc test	−	−	+	+	+	+
Carbohydrates						
Molisch test	+	+	+	+	+	+
Fehling's test	+	+	+	+	+	+
Benedicts test	+	+	+	+	+	+
Amino acids and proteins						
Biuret test	−	−	−	+	+	+
Millon's test	−	−	−	+	+	+
Ninhydrin test	+	+	+	+	+	+
Glycosides	−	+	−	+	+	+
Sterols and triterpenoids						
Liebermann–Burchard test	+	−	+	−	+	+
Salkowski's test	−	−	+	−	+	−
Tannins and phenolics						
Iodine test	−	−	−	+	+	−
Nitric acid test	+	−	−	−	+	+
Ferric chloride test	+	−	−	+	+	−
Quininoes	−	−	+	+	+	+
Saponins	−	−	−	−	+	+

+: Positive, −: Negative. *A. bettzickiana: Alternanthera bettzickiana*

dose-dependent increase in the percentage antioxidant activity for all concentrations tested. Ascorbic acid used as positive control showed scavenging activity with percentage inhibition of 81±0.02 at the maximum concentration used. An effective antioxidant concentration required to decrease the early DPPH radical concentration by 50% is known as IC_{50}, which was determined from scavenging activity plotted graph versus different concentrations of standard drug and extracts. Lower value of IC_{50} is a sign of strongest ability of extracts to act as potent DPPH radical scavengers. Highest $DPPH^{\bullet}$ scavenging activity was of hydroethanolic extract of *A. bettzickiana* followed by *A. brasiliana* with IC_{50} values of 164.25 and 182.55 µg/ml, respectively. The standard ascorbic acid showed an IC_{50} value of 78.05 µg/ml. The DPPH radical scavenging abilities of the plant extracts showed proton-donating ability, and this could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants. Thus, the hydroethanolic extracts of the tested plants had strong ability to scavenge DPPH radical.

NO scavenging assay

NO is a free radical produced in mammalian cells, have a major role in the regulation of several physiological processes, including neurotransmission, vascular homeostasis, and antimicrobial and antitumor activities. Excess production of NO is linked with several diseases [40]. The extent of inhibition of NO radical generation

Table 3: Quantitative phytochemical analysis of hydroethanolic, ethanol and water extracts of *A. brasiliana* and *A. bettzickiana*

Secondary metabolite	Extract	*A. brasiliana*	*A. bettzickiana*
Alkaloids (%)	Hydroethanolic	11.4±1.20	14.63±0.95
	Ethanol	8.8±0.63	11.7±0.60
	Water	7.76±0.42	9.7±0.56
Flavonoids (mg RU/g)	Hydroethanolic	43.6±2	60.5±1.53
	Ethanol	38.2±1.53	55.3±2
	Water	38.4±2	40.4±1.53
Total phenolics (mg GAE/g)	Hydroethanolic	34.6±2.52	47.3±0.58
	Ethanol	31.7±2.65	37.5±1.73
	Water	28.3±2.52	27.2±2.08
Tannins (mg/g)	Hydroethanolic	81.1±1.73	90.3±0.58
	Ethanol	71.3±2.65	82.7±2.31
	Water	60.5±3.51	72.9±1.73
Vitamin C (mg/g)	Hydroethanolic	75.3±4.16	85.5±4.16
	Ethanol	41.4±3.06	53.6±2.52
	Water	55.6±3.52	72.2±2
Vitamin E (mg/g)	Hydroethanolic	35.9±0.58	43.7±2.52
	Ethanol	31.7±1.15	36.9±1
	Water	37.3±1	28.5±2
GSH (mg/g)	Hydroethanolic	7.5±1	11.2±1
	Ethanol	3.1±0.58	5.2±1
	Water	7.5±0.58	8.3±0.58
Total proteins (mg/g)	Hydroethanolic	81.9±1	92.4±2.65
	Ethanol	70.5±3	83.6±2
	Water	64.3±2.31	71.4±2.31

RU: Rutin equivalent, GAE: Gallic acid equivalent. *A. brasiliana: Alternanthera brasiliana, A. bettzickiana: Alternanthera bettzickiana*

by the hydroethanolic extract from the leaves of *A. brasiliana* and *A. bettzickiana* was detected and compared with the standard ascorbic acid, and the results are shown in Table 5.

The results showed that the hydroethanolic extract of *A. bettzickiana* exhibited the maximum inhibition of 82% at a concentration of 350 µg/ ml when compared to *A. brasiliana* extract that showed somewhat lower inhibitory activity of 76% at the same concentration. The standard ascorbic acid showed maximum inhibition of 81%. The NO scavenging ability of both plant extracts was found to be concentration dependent. Highest NO scavenging activity was exhibited by a hydroethanolic extract of *A. bettzickiana* with an IC_{50} value of 104 µg/ml followed by *A. brasiliana* with an IC_{50} value of 141.07 µg/ml. The standard ascorbic acid exhibited an IC_{50} value of 20.64 µg/ml. The scavenging activity of the extracts against NO was detected by its ability to inhibit the formation of nitrite through direct competition with oxygen and oxides of nitrogen in the reaction mixture [41]. Flavonoids and phenolic compounds may be possibly responsible for NO^- scavenging activity [42].

In the present study, hydroethanolic leaf extracts of *A. brasiliana* and *A. bettzickiana* effectively scavenged NO radical, which competes with oxygen to react with NO and the formation of nitrite radical was inhibited.

Superoxide radical scavenging assay

Superoxide ion (O^{2-}) is an oxygen-centered free radical with one unpaired electron. It is a toxic product and formed as a byproduct of mitochondrial respiration, as well as several other enzymes, for example, xanthine oxidase and nicotinamide adenine dinucleotide phosphate oxidase enzyme [43]. Superoxide can be decomposed to form stronger oxidative species such as singlet oxygen and hydroxyl radicals. Superoxide ion is very harmful to the cellular components in a biological system [44]. Superoxide dismutase catalyzes neutralization of superoxide and protects against oxidative damage [45]. The decrease in absorbance at 560 nm with antioxidants indicated the consumption of SO in the reaction mixture. The extent of inhibition of superoxide radicals by hydroethanolic extracts of *A. brasiliana* and *A. bettzickiana* were analyzed using ascorbic acid as standard, and results are shown in Table 6.

The hydroethanolic extracts of *A. brasiliana* and *A. bettzickiana* exhibited potent scavenging activity for superoxide radicals in a concentration-dependent manner. The maximum scavenging activity was found at the highest concentration of 350 µg/ml, in which *A. bettzickiana* showed more activity than *A. brasiliana.* Standard drug, ascorbic acid showed superoxide radical scavenging activity with IC_{50} value of 34.88 µg/ml. *A. bettzickiana* showed more scavenging activity with IC_{50} value of 86.59 µg/ml followed by *A. brasiliana* with

Table 4: DPPH radical scavenging activity of ascorbic acid, *A. brasiliana* and *A. bettzickiana* leaf extracts

Concentration (µg/ml)	Inhibition (%)		
	Ascorbic acid	*A. brasiliana*	*A. bettzickiana*
50	47±0.57	31±0.01	35±0.02
100	53±0.00	36±0.02	40±0.00
150	58±0.00	42±0.01	49±0.01
200	63±0.00	51±0.00	58±0.02
250	68±0.00	56±0.00	62±0.01
300	73±0.01	61±0.01	66±0.01
350	81±0.02	66±0.01	73±0.01

*Mean±SD of three replicates. SD: Standard deviation, DPPH: 2,2-Diphenyl-1-picryl-hydrazyl-hydrate, *A. brasiliana: Alternanthera brasiliana, A. bettzickiana: Alternanthera bettzickiana*

Table 5: NO scavenging activity of ascorbic acid, *A. brasiliana* and *A. bettzickiana* leaf extracts

Concentration (µg/ml)	Inhibition (%)		
	Ascorbic acid	*A. brasiliana*	*A. bettzickiana*
50	53±0.02	42±0.02	45±0.01
100	59±0.05	46±0.01	49±0.00
150	67±0.04	51±0.03	55±0.02
200	74±0.01	57±0.03	61±0.01
250	79±0.02	62±0.01	68±0.01
300	85±0.02	69±0.01	75±0.03
350	81±0.02	76±0.01	82±0.02

*Mean±SD of three replicates. SD: Standard deviation, NO: Nitric oxide, *A. brasiliana: Alternanthera brasiliana, A. bettzickiana: Alternanthera bettzickiana*

124.46 µg/ml. Plant phenols may exert protective effects by scavenging superoxide, which is implicated in tissue damage and accelerated inactivation of vasorelaxing NO [46]. Polyphenols and flavonoids are well-known scavengers of superoxide radical, hydroxyl radical, and hydrogen peroxide [47]. The remarkable quantity of phenols and flavonoids detected in the quantitative estimation of *Alternanthera* leaf extracts may be responsible for free radical neutralization especially in quenching superoxide radical, singlet, and triplet oxygen. These results pointed out that the hydroethanolic extracts of *A. brasiliana* and *A. bettzickiana* were potent scavengers of superoxide radicals.

FRAP assay

The ability of *A. brasiliana* and *A. bettzickiana* leaf extracts to reduce ferric ions was shown in Table 7.

Ferric reducing the antioxidant potential of *A. brasiliana and A. bettzickiana* extracts were estimated by their capability to reduce tripyridyl triazine (TPTZ)–Fe (III) complex to TPTZ–Fe (II). Considerable FRAP activity of standard and extracts as represented in Table 7 confirmed their reducing capacity. There was a concentration-dependent increase in the antioxidant activity of both plant extracts. At high concentration of 350 µg/ml, *A. brasiliana and A. bettzickiana* extracts exhibited maximum activity. Antioxidants play an important role as reducing agent as they break the chains of free radicals by donating hydrogen atom [48]. The result of our study was comparable with the study of Benzie and Szeto [49] who found out that higher the phenolic content, higher would be the reducing power of the extract and these two have the strong correlation with each other. Rice-Evans *et al.* [50] stated that the phenolic compounds have the redox potential, hence, have the reducing properties, and can donate hydrogen atom as well as quench the singlet oxygen.

TAC

The total antioxidant activity of leaf extract was measured to evaluate both water-soluble and fat-soluble antioxidants totally. The principle of this assay involves the activity of an antioxidant compound that leads to a reduction of the hexavalent form of molybdenum [Mo (VI)] to the pentavalent form [Mo (V)] and the formation of a green phosphate/Mo (V) complex at acidic pH and at a higher temperature. This is spectrophotometrically measured at 695 nm. The intensity of green phosphomolybdenum complex gives the measure of total antioxidants present in the sample [51]. The results of total antioxidant activity in the hydroethanolic leaf extracts of *A. brasiliana* and *A. bettzickiana* are presented in Table 8.

The leaf extract of *A. bettzickiana* exhibited higher (191 mg/g) total antioxidant activity than *A. brasiliana* (179 mg/g). In both leaf extracts, there was a dose-dependent increase in total antioxidant activity. Many flavonoids and related polyphenols contribute significantly to the phosphomolybdate scavenging activity of medicinal plants [52,53]. The present study was in agreement with the above findings and indicated that the presence of significant levels of non-enzymic antioxidants, namely, flavonoids and polyphenols in leaf extracts of *Alternanthera* plants might contribute to the phosphomolybdate scavenging activity which was measured as TAC.

Reducing power assay

Reducing power assay served as a significant reflection of the antioxidant activity [54]. Reducing power is evaluated by the transformation of Fe^{3+} to Fe^{2+} in the presence of the extract that possesses reducing property [55]. Reducing the power of standard ascorbic acid, *A. brasiliana* and *A. bettzickiana* extracts were shown in Table 9.

The reducing power of both leaf extracts was found to be concentration dependent. The maximum reducing power was found at the highest concentration (350 µg/ml) in which *A. bettzickiana* showed more activity than *A. brasiliana.* Compounds with reducing power indicated that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes so that they can act as primary and secondary antioxidants [56]. The antioxidants, present in the plant

Table 6: Superoxide radical scavenging activity of ascorbic acid, *A. brasiliana* and *A. bettzickiana* leaf extracts

Concentration (µg/ml)	Inhibition (%)		
	Ascorbic acid	*A. brasiliana*	*A. bettzickiana*
50	52±0.02	41±0.02	44±0.01
100	56±0.03	48±0.02	51±0.02
150	66±0.02	53±0.01	59±0.02
200	73±0.02	64±0.01	67±0.01
250	77±0.02	69±0.00	73±0.02
300	84±0.03	73±0.02	78±0.02
350	89±0.02	79±0.02	83±0.01

*Mean±SD of three replicates. SD: Standard deviation

Table 7: FRAP assay of ascorbic acid, *A. brasiliana* and *A. bettzickiana* leaf extracts

Concentration (µg/ml)	Optical density (593nm)		
	Ascorbic acid	*A. brasiliana*	*A. bettzickiana*
50	0.56±0.01	0.31±0.01	0.34±0.01
100	0.62±0.02	0.39±0.02	0.45±0.02
150	0.69±0.02	051±0.00	0.54±0.01
200	0.76±0.02	0.59±0.01	0.63±0.01
250	0.80±0.01	0.63±0.00	0.68±0.01
300	0.85±0.00	0.73±0.03	0.77±0.03
350	0.90±0.01	0.81±0.02	0.85±0.03

*Mean±SD of three replicates. SD: Standard deviation, FRAP: Ferric reducing antioxidant power, A. brasiliana: Alternanthera brasiliana, A. *bettzickiana: Alternanthera bettzickiana*

Table 8: TAC of *A. brasiliana* and *A. bettzickiana* leaf extracts

Concentration (µg/ml)	TAC	
	(mg of ascorbic acid/g of *A. brasiliana*)	(mg of ascorbic acid/g of *A. bettzickiana*)
50	18	21
100	56	61
150	95	101
200	112	136
250	179	191

TAC: Total antioxidant capacity, *A. brasiliana: Alternanthera brasiliana, A. bettzickiana: Alternanthera bettzickiana*

Table 9: Reducing power of *A. brasiliana* and *A. bettzickiana* leaf extracts

Concentration (µg/ml)	Absorbance at 700 nm		
	Ascorbic acid	*A. brasiliana*	*A. bettzickiana*
50	0.119±0.000	0.107±0.000	0.111±0.000
100	0.123±0.000	0.113±0.001	0.118±0.000
150	0.129±0.000	0.117±0.002	0.121±0.000
200	0.133±0.000	0.122±0.002	0.126±0.000
250	0.152±0.000	0.137±0.001	0.142±0.000
300	0.162±0.000	0.148±0.002	0.156±0.000
350	0.185±0.001	0.160±0.002	0.169±0.005

*Mean±SD of three replicates. SD: Standard deviation, *A. brasiliana: Alternanthera brasiliana, A. bettzickiana: Alternanthera bettzickiana*

extracts reduce the ferric cyanide complex (Fe^{3+}) to ferrous cyanide and form Fe^{2+} [57]. The presence of higher amount of reductones in the hydroethanolic extract of *A. brasiliana* and *A. bettzickiana* that impart antioxidant action by donating a hydrogen atom may be responsible for the reducing ability.

CONCLUSION

The qualitative and quantitative phytochemical analysis of various extracts of leaves of A. brasiliana and A. bettzickiana revealed that hydroethanolic extract as a rich source of biologically important phytonutrients. Free radical scavenging and in vitro antioxidant studies showed better scavenging ability of hydroethanolic leaf extracts of A. *bettzickiana* than *A. brasiliana*. Both plants might be a good source of dietary antioxidants which play an important role in the prevention of diseases associated with oxidative stress. To increase the antioxidant potential of the plants, the pure active compound that has the ability to scavenge free radicals should be isolated and could be used in curing many oxidative stress-related diseases such as cancer, cardiovascular, and other chronic diseases.

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AUTHORS' CONTRIBUTIONS

Mrs. Kasthuri OR contributed in performing the experiment, data compilation and wrote the first draft of the manuscript. Dr. Ramesh B involved in corrections in the manuscript and overall management of the study. Both authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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