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Analysis of proximate, phytochemical, elemental compositions and antioxidant property of leaf of *Alternanthera brasiliana* (l.) kuntze

Abstract

Alternanthera brasiliana is a medicinal plant which is reported to have many health benefits. In the present study, the qualitative phytochemical analysis of three solvent (hexane, chloroform and methanol) extracts of A. brasiliana leaf revealed the presence of carbohydrates, alkaloids, saponins, flavanoids, phytosterols and phenols, tannins and proteins. The shade dried leaf powder was subjected to proximate and elemental analysis. The proximate compositions such as the moisture 68%, carbohydrate 9.5%, crude protein content was 4.3%, crude fat 0.4% and the energy or calorific value was found to be 58.8Kcal/100g. The elemental composition such as carbon (C), hydrogen (H), nitrogen (N) and sulfur (S) was quantified using CHNS analyzer and the corresponding values are 384.7mg/g, 59.2mg/g, 47.8mg/g and 6.6mg/g respectively. The other elements such as calcium (Ca), potassium (K), magnesium (Mg), phosphorus (P), copper (Cu), zinc (Zn) and sodium (Na) are also found in the leaf of A. brasiliana and were detected using Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES) and the corresponding values obtained are 18mg/g, 21.1mg/g, 9.1mg/g and 1.4mg/g; 0.01999mg/g, 0.28727mg/g and 0.94332mg/g respectively. The antioxidant activity of methanolic extract of A. brasiliana leaf on DPPH radical was 64.08% at a concentration of 1000µg/ml and the IC50 value of methanol leaf extract was 572.85mg/ml. This study was aimed to ascertain the presence of important phytochemicals, proximate composition, macro, micro and other essential elements and the presence of antioxidants in A. brasiliana leaf samples to confirm the possible use as fodder additive.

Keywords: alternanthera brasiliana, proximate, antioxidant, energy value, elemental analysis, tannin

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Introduction

Alternanthera brasiliana (L.) Kuntz (Amaranthaceae) is an indigenous herb found in Brazil and is commonly known as penicillin or Brazilian joy weed, which grows easily on poor and deforested soil. It is described as perennial, prostrate and branchy, presenting a circular to polygonal stem, long internodes and swollen nodes at which opposite leaves attach. The inflorescence is cymes, composed of hermaphrodite, actinomorphous and monocyclic flowers.[1]

A. brasiliana possesses medicinal property and this plant has gained great importance globally today as it acts as potent nutritional and therapeutic agents capable of curing man's ailments. The chemical composition diversity in plants includes various bioactive compounds that are beneficial to humans like vitamins, nutrients, antioxidants, anticarcinogens, and many other compounds with medicinal value.[2] Bioactive compounds or phytochemicals of plant origin acts along with nutrients and dietary fibre to protect against various ailments.[3,4] Phytochemicals can be categorized into various groups that are as polyphenols, organosulfur compounds, alkaloids and nitrogen-containing compounds. The polyphenols are some of the most studied compounds and can be further divided into flavonoids (including flavonols, flavones and catechins; flavonones, anthocyanidins and isoflavones), phenolic acids and stilbenes; coumarins and tannins.[5] Their functions and mechanism of actions include antioxidant activity, hormonal action, stimulation of enzymes, interference with DNA replication and antibacterial properties.[6]

A. brasiliana is popularly used against inflammation, cough and diarrhoea in Brazilian medicines.[7] The extract of *A. brasiliana* leaves exhibited antinociceptive, anti-microbial, anti-inflammatory, antiherpe simplex virus activity and with the influence of different kinds of light and also produces compounds with possible analgesic action.[8] It constitutes various nutritional and antinutritional components in addition to the therapeutically important secondary metabolites.[9] They are also used in animal feed as the growth promoters and for enhancement of productivity.[10] The use of plant derived antioxidants results in lesser side effects and is widely used as dietary supplements. Natural antioxidants are considered as safe and cause fewer adverse reactions than synthetic antioxidants.[11] A large number of plants have been screened as a viable source of natural antioxidants such as tocopherol, vitamin C, carotenoids, and phenolic compounds which are responsible for maintenance of health.[12] Knowledge of the phytochemical constituents present in *A. brasiliana* will be very useful for the maximum usage of this plant in medicine.

Proximate analysis of edible plant and vegetables plays a crucial role in assessing their nutritional significance.[13] Analysis of mineral elements is most important to understand the pharmacological and nutritional values of medicinal plants. The elements present in the food at major, minor and trace levels are vital for human wellbeing and their ingestion in excess or limited amount can cause severe health problems.[14,15] Human body is in need of both metallic and non-metallic elements for healthy growth, development and proper functioning of the body. Hexane leaf extract of *A. brasiliana* exhibited

good antibacterial activity against *E. coli* with an inhibition zone of 22.33mm.[16] Aqueous and ethanol extract of *A. brasiliana* leaf is able to inhibit human mitogen induced lymphocyte proliferation without any toxic effect.[17,18] *Alternanthera philoxeroides* has been found to be a rich source of iron and may be used as an ingredient in salad. This species may also be used for the production of methane gas and tertiary filtration system for domestic sewage and this signifies the nutritional and economical potential of this genus, *Alternanthera*.[19] Hence, the present study was undertaken to investigate the proximate, phytochemical, elemental compositions and antioxidant activity of *A. brasiliana* leaf for a possible use as fodder additive.

Materials and methods

Collection and preparation of plant material

Fresh and healthy leaves of *A. brasiliana* were collected from Cherthala, Alappuzha district, Kerala state, India. The plant material was identified by Dr. Shaji P.K., Scientist, Environmental Resources Research Centre (ERRC), P.B. No. 1230, P.O. Peroorkada, Thiruvananthapuram, Kerala state, India. The plant materials were initially cleaned, dried under shade and then pulverized to coarse powder in an electric grinder. The powder was then stored in airtight bottles for further studies.

Proximate analysis of leaf samples

Moisture, crude protein (crude protein included both true protein and non-protein nitrogen), carbohydrate and crude fat of shade dried leaf powder were determined following the methods described by the Association of Official Analytical Chemists.[20]

Chemicals and reagents

Butylated hydroxyl anisol (BHA), 2.2 Diphenyl-1-picryl hydrazyl (DPPH), sodium nitrate, sodium hydroxide, H_2SO_4 , acetic acid, NaOH, ninhydrin and solvents (hexane, chloroform and methanol) were purchased from Hi Media Laboratories Pvt. Limited, Mumbai, India. Aluminium chloride, Folin ciocalteu reagent and other reagents were purchased from Merck Limited, Mumbai, India. All the chemicals and reagents used were of analytical grade and were prepared in deionized water.

Phytochemical analysis

Leaf extracts of hexane, chloroform and methanol were subjected to various phytochemical analyses using standard methods.[21–23]

Carbohydrates

Molisch's test was performed to detect carbohydrates. A few drops of alcoholic alpha naphthol solution were added to the extracts and added 1ml of concentrated sulphuric acid along the sides of the test tube. The formation of violet ring at the junction of the liquids indicated the presence of carbohydrates.

Alkaloids

The extract was mixed with 2ml of Wagner's reagent and the formation of reddish brown colored precipitate indicated the presence of alkaloids.

Glycoside

Keller-Kelliani test was performed to detect cardiac glycoside. To the 5ml of extract, added 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1ml of concentrated H_2SO_4. A brown ring of the interface indicated a deoxy sugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout the thin layer.

Steroids

Steroids were tested by the reaction of Liebermann. 10ml of ethanolic extract was evaporated and the residue was dissolved in 0.5ml of hot acetic anhydride and added 0.5ml of chloroform. Then the mixture was treated with the reagent of Libermann Burchardt. The appearance of blue-green ring at the interphase denotes a positive reaction.

Saponins

Foam test was performed to test the presence of saponins. To the 2ml of the extract, added 6ml of water in a test tube and was shaken vigorously, then observed for the formation of persistent foam that confirms the presence of saponins.

Flavonoids

Alkaline reagent test was performed to test the presence of flavonoids. The extracts were mixed with 2ml of 2% solution of NaOH. An intense yellow color was formed which turned colorless on addition of few drops of diluted acid which indicated the presence of flavonoids.

Phytosterols

Salkowski test was used to detect the presence of phytosterols. To the 2ml of aqueous extract, 2ml of chloroform and 2ml of concentrated H_2SO_4 was added. The solution was shaken well. As a result the chloroform layer turned red and acid layer showed greenish yellow fluorescence.

Triterpenoids

Liebermann Burchard's test was performed to detect the presence of triterpenoids in the leaf sample. The extract was treated with chloroform, filtered and a few drops of acetic anhydride was added to the filtrate, boiled and cooled. Then concentrated sulphuric acid was added. Formation of deep red color indicates the presence of triterpenoids.

Phenols and tannins

The extracts were mixed with 2ml of 2% solution of $FeCl_3$. A blue green or black coloration indicated the presence of phenols and tannins.

Proteins

Ninhydrin test was performed to detect the presence of proteins in the extracts. Crude extract when boiled with 2ml of 0.2% solution of ninhydrin, violet color will appear which indicate the presence of amino acids and proteins.

Determination of energy or calorific value

The total energy value in the leaf of *A. brasiliana in* Kcal/100g was estimated using the formula of FAO.[24] The equation for energy determination was Energy value (Kcal/100g)=[% crude proteinx4.0]+[% crude fatx9.0]+[% carbohydratex4.0]

Elemental analysis

Elements present in leaf samples, such as carbon (C), hydrogen (H), nitrogen and sulfur (S) were analyzed using CHNS analyzer (Elementar Vario EL III). Samples are dispensed into small tin capsules, which are carbonized at high temperatures (>900°C) for a few minutes. The contents of C, H, N and S are oxidized and converted into gaseous forms, which are registered by the integrator connected to the analyzer. Elements such as Calcium (Ca), Potassium (K), Magnesium (Mg), Phosphorus (P), Copper (Cu), Zinc (Zn) and Sodium (Na) were estimated using Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES, Thermo Electron IRIS INTREPID II XSP DUO). 1g of leaf powder was digested in 10ml of ultrapure metal free nitric acid in a microwave digester (Milestone). After digestion, the content was diluted to 25ml with distilled water. The microwave digested sample was aspirated into ICP-AES to detect and quantify the elements. The calibration standards were prepared by diluting the stock multi-elemental standard solution (1000mg/l) in nitric acid.

Preparation of leaf extract for antioxidant assays

The powdered leaf samples (30g) were suspended in 250ml methanol and kept for 72hours. After 72hours, the extract was filtered using a What man No1 filter paper and the filtrate was concentrated under reduced pressure using rotary evaporator (IKA RV 10 digital, Germany). The dried extracts were then stored at 4°C for further assays**.**

DPPH Radical Scavenging Assay

The antioxidant activity was evaluated according to the scavenging activity of stable radical DPPH. The DPPH free radical scavenging activity of methanolic leaf extracts at different concentrations were performed by using method based on the reduction of methanolic solution of colored free radical 2.2 Diphenyl -1-picryl hydrazyl by free radical scavenger.[25] BHA was used as the standard antioxidant. 0.1ml solution of different concentration (50, 100, 250, 500, 750 and 1000µg/ml respectively) of extract was added to 1.4ml of DPPH (0.05mg/ml) and kept in dark for 30min. The absorbance was measured at 517 nm using spectrophotometer (Shimadzu UV 1800) and the percentage inhibition was calculated by using the following equation.

Percentage inhibition (%)=(A0 -A1)/A0)×100

Where: A0 is the Absorbance of control and A1 Absorbance of test.

Results and discussion

Proximate analysis

The moisture content in the leaf sample was found to be 68%. The crude protein (crude protein included both true protein and non-protein nitrogen) content was 4.3%. The presence of crude proteins plays an important role in organoleptic properties of food apart from their nutritional significance as a source of amino acids.[26]

The carbohydrate content in the leaf sample was 9.5% and crude fat 0.4% respectively. The energy value was found to be 58.8Kcal/100g. Analysis of the edible portion of *A. brasiliana* gave the following values: moisture 77.4%, protein 5%, fat 0.7%, carbohydrates 11.6% minerals 2.5g/100g, calcium 510.0mg/g and calorific value of 73 kcal.[27] The values expressed are similar to the observations made in the present study.

The carbohydrate content is a source of energy and is required for efficient oxidation of fats.[28] Carbohydrate is also beneficial since it constitutes a major class of naturally occurring organic compounds that are essential for the maintenance of plant and animal life and also provide raw materials for many industries.[29]

Phytochemical screening

The preliminary qualitative phytochemical screening of hexane, chloroform and methanol leaf extracts indicated the presence of alkaloids, phenols and flavonoids; saponins, tannins and phytosterols; protein and carbohydrates (Table 1). Carbohydrate, phenols and tannins were commonly present in hexane, chloroform and methanol leaf extracts. Carbohydrates, fats and proteins play a vital role in satisfying human needs for energy and life processes.[2] Phytochemicals or secondary metabolites usually occur in complex mixtures that differ among plant organs and stages of development.[30,31] The hexane leaf extract showed the presence of phytochemical substances such as phenols, flavanoids, alkaloids, tannins, phytosterols, saponins, carbohydrate. Phenolic compounds detected in this plant might be responsible for the antioxidant property. Phenols are the important plant derived bioactive compounds because of their ability to scavenge free radicals due to their hydroxyl groups and it may directly contribute to their antioxidant potential and antibacterial activities.[11] Flavanoids and saponins were not found in chloroform and methanolic extract (Table 1). Glycosides, steroids and triterpenoids were absent in all the three solvent extracts studied.

Table 1 Phytochemical constituents of *A. brasiliana*

Sl. No.	Tests	Method	Presence (+) or absence (-) of Phytochemicals		
			Hexane	Chloroform	Methanol
1	Carbohydrates	Molisch's test	+	+	+
2	Alkaloids	Wagners test	+	–	+
3	Glycosides	Keller killiani test	-	-	-
4	Steroids	Liebermann reaction	-	-	-
5	Saponins	Foam test	+	–	–
6	Flavanoids	Alkaline reagent test	+	–	–
7	Phytosterols	Salkowski test	+	+	+
8	Triterpenoids	Liebermann Burchard's test	-	-	-
9	Phenols	Ferric Chloride test	+	+	+
10	Tannins	Ferric Chloride test	+	+	+
11	Proteins	Ninhydrin test	-	-	+

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Elemental analysis

Elemental analysis was performed using Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES). It is one of the most common techniques for elemental analysis. Its high specificity, multi element capability and good detection limit results in the use of this technique in a very large variety of applications.

The elemental analysis revealed the presence of macro, micro and other essential elements. Calcium, potassium and magnesium; phosphorus, nitrogen and carbon; sulphur and hydrogen were found in appreciable levels. Elements like copper, zinc and sodium is present in lower quantities (Table 2). Carbon is found to be the highest element accounted for 384.7mg/g followed by H 59.2mg/g, N 47.8mg/g and K 21.1mg/g respectively. Carbon, hydrogen and nitrogen are the basic element and most important in living organisms. Nitrogen is an essential building block of amino acids and nucleic acids.

Table 2 Elemental composition of A. brasiliana leaf

Elements	Concentration (mg/g)
C	384.7
H	59.2
N	47.8
S	6.6
Ca	18
K	21.1
Mg	9.1
P	1.4
Cu	0.01999
Zn	0.28727
Na	0.94332

A. brasiliana contains calcium with 18mg/g, sulphur 6.6mg/g, zinc 0.28727mg/g and magnesium 9.1mg/g respectively. The values obtained for other elements are given in Table 2. Salvador et al.[32] performed the elemental analysis of *A. brasiliana* (total plant) and reported the presence of P, K, Ca, Zn and Cu and this result is in agreement with the present findings.[32] Magnesium plays crucial role in lipid membrane stabilization, replication and metabolic processes.[33] Magnesium is essential for all biosynthetic processes including glycolysis, formation of cyclic AMP, energy dependent membrane transport and transmission of the genetic code. Magnesium is also required for maintenance of electrical potentials of nerve and muscle and for the transmission of signals across neuromuscular junctions. Potassium and sodium both are essential and they play crucial role in the cellular homeostasis.[34] Calcium is main component in bone and helpful for regulating skeletal and cardiac muscles contractions.[35] Calcium also plays a role in several process such exocytosis, neurotransmitter release and muscle contraction in smooth muscle. Zinc is most important and plays a role in the structure of proteins as well as in enzymatic catalyst.[36]

Minerals present in the plants play a major role in regulating many vital physiological processes in the body of animals which feed them such as regulation of enzyme activity, skeletal structures, neuromuscular irritability and clotting of blood.[37] Deficiency of any one of the essential minerals can lead to chronic metabolic disorders and can hamper the health of the organism which feed them.

Antioxidant activity

The scavenging ability of *A. brasiliana methanolic* extract on DPPH radical was 64.08% at a concentration of 1000µg/ml where as the standard BHA exhibited 95.69% radical scavenging ability at 1000µg/ml concentration (Figure 1). The IC_{50} value of methanol leaf extract was 572.85mg/ml, where as the standard antioxidant BHA showed an IC_{50} value of 123.23mg/ml. Osmund et al.[38] recorded 99.5% DPPH radical scavenging activity at a concentration of 0.1µg/ml on *A. braziliana* leaves.[38]

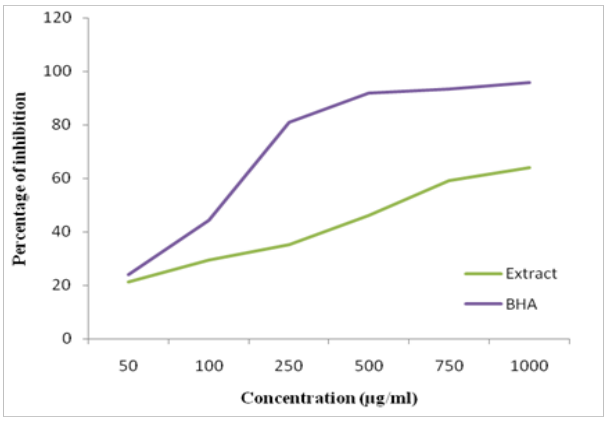

Figure 1 Antioxidant activity of methanol leaf extract of *A. brasiliana*.

The synthetic antioxidant (BHA) was commonly used in the food industries may not be as safe as it was presumed earlier. As the BHA may be carcinogenic, it is important to look for new sources of natural antioxidants from fruits and vegetables.[27] Butylated hydroxyanisole (BHA, 3-tert-butyl-4-hydroxyanisole) and butylated hydroxytoluene (BHT, 3, 5-di-tert-butyl-4-hydroxytoluene) are the common synthetic antioxidants used in the food industries for human consumption. They are commonly added into food products such as vegetable oils and other food items to extend their shelf life and prevent oxidative damages.[39]

Antioxidants are compounds which can scavenge the free radicals produced due to various biochemical processes in the human body and prevent the damage induced by them. They can effectively reduce oxidative damage of human body from reactive oxygen species (ROS) by neutralizing the free radicals before they can attack the cells and prevent damage to lipids, proteins and enzymes, carbohydrates and DNA.[40] The effect of antioxidants on DPPH radical was thought to be due to their hydrogen donating ability to the free radicals and reducing it to nonreactive species.[41] Flavonoids and phenolic compounds widely distributed in plants which have been reported to exert multiple biological effect, including free radical scavenging abilities, anti-inflammatory, anti carcinogenic etc.[42] The presence of phenols and flavonoids were also revealed in the present investigation. The search for identifying natural antioxidants in plants is the most happening area in research as most natural antioxidants are multifunctional, safe and with lesser side effects.[43] In addition to this, Biavatti et al.[44] used *A. brasiliana* extracts (180ml/200kg feed) as a broiler feed additive and this improved broiler performance from 14 to 21days.[44] The findings in the present study reveal that the leaf of *A. braziliana* can be used as a potential candidate as a fodder additive.

Conclusion

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The present study reveals the presence of important phytochemicals, moisture, carbohydrates and crude proteins, crude fat and considerable calorific value in the plant leaf. The elements such as carbon, hydrogen, nitrogen and sulfur; calcium, potassium, magnesium and phosphorus; copper, zinc and sodium are present in appreciable levels. The methanolic extract showed antioxidant property and this reaffirms the medicinal and nutritive potential of *A. brasiliana.* Because of the presence of above said properties and nutritional factors *A. brasiliana* leaves can be considered as a potential candidate for fodder additive.

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None.

Conflict of interest

The author declares no conflict of interest.

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