ISSN 2583– 0260 (online) *ISSN 2394 -7837 (print)*

PHYTOCHEMICAL, ANTI-OXIDANT AND HPTLC ANALYSIS OF ALTERNANTHERA BRASILIANA (L.) KUNTZE

Pillai Lakshmi Sreekumar* and Vidhu R.

Received: 7/3/2024 **Revised: 22/5/2024** **Accepted:25/5/2024** **Published: 30/12/2024**

Abstract

Medicinal plants have wide spread properties due to the presence of phytochemicals. The aim of the present study is the systematic study on the phytochemical composition and the biological activities to validate the pharmaceutical potential of *Alternanthera brasiliana*. *Alternanthera brasiliana* (L.) Kuntze is often referred to as "terramycin," "penicillin," or "benzetacil." It is used in traditional medicine to treat infections; the infusion of its leaves is used as a digestive, depurative, and diuretic; and the maceration of the entire plant is used to treat constipation. In the present study the whole plant was shade dried and ground to fine powder followed by organoleptic, physico-chemical and phytochemical analysis. HPTLC was conducted for determining the active components present in the plant. Antioxidant activity of the plant extract was tested using DPPH and Hydrogen peroxide scavenging assays. The organoleptic study was conducted in the plant powder in order to find out the colour, taste, odor and texture of *Alternanthera brasiliana* powder. The plant powder was subjected to soxhlet extraction using methanol. Phytochemical screening of the plant extract was done according to the standard biochemical procedures. The qualitative and quantitative analysis determined the presence of various phytochemicals such as tannins, saponins, flavonoids, alkaloids, phlobatannins, simple phenolics, coumarins, quinones, flavanols, lignin in the plant extract. The plant possessed good antioxidant activity comparable to the standard ascorbic acid. Determination of the natural antioxidants from plant extracts will help to develop new drugs for antioxidant therapy. However, further studies are needed to elucidate the *in vivo* potential of *Alternanthera brasiliana* in the management of human diseases.

Key words: Phenolics, Flavanols, Digestive, Accessible

Introduction

Traditional herbal medicine (or alternative herbal medicine) has played an essential role as a source of primary health care for many globally (Maroyi and Cheikhyoussef, 2015). Traditional medicine has remained the most affordable and easily accessible source of treatment in primary health care system of poor communities where alternative therapy is the major means of medical treatment in such communities (Yingar & Yewhalaw, 2007). According to Azaizeh et al (2003) reported that about 80% of the world population depend on traditional medicine for their health care. Traditional medicines are usually cheaper than modern medicines and probably the only natural remedies available and accessible in the remote rural communities in developing countries (Popovicetal., 2016). Traditional herbal medicine has been used to prevent many diseases. The term pharmacognosy was first used between 1811 and 1815, and originally referred to ‘‘materia medica’’, the knowledge of drug materials or pharmacology.It is derived from two Greek words, pharmakon (a drug) and gignosko (to acquirea knowledge of) (Evans 1996). Later on, pharmacognosy became restricted to that branch of pharmacy investigating ‘‘medicinal substances from the plant, animaland mineral kingdoms in their natural, crude, or unprepared state, or in the form of such primary derivatives as oils, waxes, gums, and resins’’ (Hocking 1997). In a further attempt to update the scope ofthis field in a manner consistent with scientific activities ongoing atthe beginning of the 21st century, pharmacognosy has recently been defined as ‘‘a molecular science that explores naturally occurring structure–activity relationships with a drug

Post Graduate Department of Botany and Research Centre
N.S.S. College, Pandalam, Pathanamthitta, Kerala, India (Affiliated to University of Kerala, Thiruvananthapuram, Kerala, India). *email:* abrus09@gmail.com (*Corresponding author)

potential'' (Bruhn &Bohlin 1997).The American Society for Pharmacognosy defines pharmacognosy as "the study of the physical, chemical, biochemical, and biological properties of drugs, drug substances, or potential drugs or drug substances of natural origin, and the search for new drugs from natural sources"(Sarker ,2012).

Phytochemicals are by-products of primary metabolic functions of the plant, otherwise called the secondary metabolites (Richter, 1978). They are produced and used by the plants forprotection and repair processes within the natural environment (Bako et al., 2005). According to Heldt (2005), most of these phytochemicals are produced through biosynthesis in the metabolic pathways.Secondary metabolites have both a defensive roleagainst herbivory, pathogen attack and inter-plant competition and an attractant role towards beneficial organisms such as pollinators or symbionts (Kaufmann et al., 1999).

Oxidative stress is hazardous to the body because it causes peroxidation of membrane lipids, which leads to membrane integrity loss and cell death, as well as denaturation of proteins such as enzymes, ion channels, and DNA strand breaks. As a result, they may be associated with specific pathophysiological ailments such as arthritis, hemorrhagic shock, coronary artery disease, cataract, cancer, AIDS, and age-related degenerative brain problems. Antioxidants are substances that can reduce oxidative stress and the occurrence of oxidant-related disease disorders(Murota and Terao 2003).

High-performance thin-layer chromatography (HPTLC) is an efficient, sophisticated and automated form of the thin-layer chromatography (TLC) with better and advanced separation efficiency and detection limits (Saibaba and Shanmuga, 2016). It is faster, easier and more flexible as compared to any other chromatographic technique (Eike and Anne 2006). Modern high-performance TLC (HPTLC) is an efficient instrumental analysis, and optimised quantitative HPTLC using a densitometric evaluation can produce results analogous to those obtained with gas chromatography (GC) and high performance liquid chromatography (HPLC) (Wagner and Bladt, 2001 and Medic-Saric etal.,2008).Thus, HPTLC 'fingerprint analysis' may be a powerful tool for the quality control of raw plant material and may be an alternative technique, particularly in the analysis of crude plant extracts. An improvement over conventional TLC, HPTLC is an instrumental technique where by special plates and instrumental resources for sampling are used and the quantitative evaluation of separations is aided by densitometry(Nile andPark, 2014).

The inflorescence is cymes, composed of hermaphrodite, actinomorphous and Monocyclic flowers (Duarte and Debur, 2004). *Alternanthera brasiliana* is popularly used against inflammation, cough and diarrhoea in Brazilian medicines (Brochadoetal.,2003). It constitutes various nutritional and anti-nutritional components in addition to the therapeutically important secondary metabolites (Dingman, 2002).

The aim of the present work is therefore a systematic study on the phytochemical composition and the biological activity to validate the pharmaceutical potential of *Alternanthera brasiliana*.

Materials and Methods

Plant material

The plant selected for the study, *Alternanthera brasiliana* was collected from Punalur, Kollam. The whole plant was used for the present study.

A Powder analysis: Fresh plant of *Alternanthera brasiliana* was collected in a polythene bag. The collected materials were washed under tap water to remove dirt. Then it was shade dried and powdered in a mixer grinder and sieved with a fine mesh sieve. The powder was then used for the organoleptic study, physicochemical analysis and solvent extraction.

1. Organoleptic study

Organoleptic (literally "impression on the organs") refers to the evaluation by means of the organs of sense and includes the macroscopic appearance of the plant material, its color, odor, and taste, occasionally the sound of 'snap' of its fracture and the 'feel' of the powder to the touch (Wozniak et al., 1997). The plant powder characteristics like the color, odor, taste and nature were evaluated.

2. Physico-chemical characterization

Different physicochemical parameters were determined according to the official methods and guidelines on quality control for medicinal plant materials.

a. Loss on drying (Indian Pharmacopoeia, 1992)

b. Foaming index (WHO, 1992)

c. Swelling index (WHO, 1992)

d. Foreign matter (Indian Pharmacopoeia, 1996)

e pH (Iqbal et al., 2010)

B. Phytochemical Screening

1. Preparation and yield of extract (Indian pharmacopoeia, 1996)

About 15 g of the powdered plant material was subjected to extraction by Soxhlet apparatus using 100 ml methanol. The extract was concentrated under reduced pressure and preserved in refrigerator until further use. The percentage of the crude extract was determined using the following equation.

Percentage yield (%) = (weight of the crude extract/weight of the sample) X100

2. Qualitative analysis

Different phytochemical constituents (15) were tested using standard biochemical procedures (Harborne, 1973).

3. Quantitative analysis

a. Determination of Alkaloids (Harborne, 1973)

b. Determination of total flavonoids (Harborne and Williams,2000)

4. Biological activity—Antioxidant assay

a. DPPH (1–1-diphenyl–2–picryl hydrazine) Free Radical Scavenging Assay (Mensor et al., 2001)

b. Hydrogen Peroxide Scavenging Assay (Ruch et al., 1989)

Statistical analysis

Experimental results were analyzed statistically by Probit (IC50 values) and Regression analysis.

5. HPTLC (High Performance Thin Layer Chromatography)

The extracts were applied as different tracks of different concentrations of width 8 mm each on silica gel 60 F254 pre-coated aluminium sheets through CAMAG micro litre syringe using Automatic TLC Sampler 4 (ATS4). After sample application the plate was introduced vertically in a CAMAG developing chamber (10 cm x 10 cm) pre-saturated with the mobile phase selected. The developed chromatogram was air dried to evaporate solvents from the plate and the plate was kept in CAMAG Vizualizer and the images were captured under UV light at 254 nm and 366 nm. The plate was scanned at 254 nm and 366 nm using TLC Scanner 4 and the finger print profiles were documented. The Re-values and finger print data were recorded with win CATS software associated with the scanner. The plate was derivatized using vanillin-sulphuric acid reagent, heated at 105° C by placing on CAMAG TLC plate heater till the colour of the bands appeared. Then the plate was visualized under white light and the chromatograms were documented. The plate was scanned at 575 nm and the Rf values and finger print data were documented (Sisodiya and Srivastava,2017).

Results

A. Powder analysis

1. Organoleptic study

The organoleptic characteristics like color, odor, taste and texture of *Alternanthera brasiliana* were determined (Table 1).

2. Physico– chemical characterization A total of five phytochemical parameters were evaluated in *Alternanthera brasiliana.* The amount of matter was low in *Alternanthera brasiliana.*

The moisture content was reported to be in low amount in the plant. Foaming index was found to be more than 100 units indicating the presence of saponins. No considerable swelling was observed due to the absence of gums and mucilage in the plant powder. The value of pH indicated the basic nature of the powder (Table 2).

Table 1. Organoleptic characters of *Alternanthera brasiliana*

Characters	*Alternanthera brasiliana*
1. Color	Olive green
2. Odor	Pungent
3. Taste	Bitter
4. Texture	Rough

Table 2. Physio- chemical characters of *Alternanthera brasiliana*

Parameters	Values
Foreign matter	0.61%
Loss on drying	6.50%
Swelling index	Nil
Forming index	>100ml
pH	7.5

B. Phytochemical Screening

1. Yield of the extract

The methanol extract of *Alternanthera brasiliana* was prepared by Soxhlet extraction. The yield of the methanol extract was 4.2%.

2. Quantitative analysis

A total of 15 phytochemicals were qualitatively assessed. Terpenoids, glycosides, acid, steroids, gum, and mucilage were absent in the plant extract. Tannins, saponins, flavonoids, alkaloids, phlobatannins, Simple phenolics, Coumarins, Quinones, Flavanols, Lignin were detected in methanol extract of *Alternanthera brasiliana* (Table 3).

3. Quantitative analysis

The quantitative estimation of two phytochemicals was performed in the methanol extract of the plant *Alternanthera brasiliana* using conventional procedures (Table 4). Flavonoids and alkaloids were found to be in considerable amounts in the extract.

Table 3. Phytochemicals tested in *Alternanthera brasiliana*

SL. No.	Phytochemicals	Present / Absent
1	Tannins	+
2	Saponins	+
3	Flavonoids	+
4	Alkaloids	+
5	Terpenoids	-
6	Phlobatannins	+
7	Glycosides	-
8	Simple phenolics	+
9	Coumarins	+
10	Quinones	+
11	Acids	-
12	Flavanols	+
13	Lignin	+
14	Steroids	-
15	Gums and mucilage	-

Table 4. Quantitative analysis in *Alternanthera brasiliana*

Phytochemicals	Amounts (mg/g)
Flavonoids	5.62
Alkaloids	1.48

4. Antioxidant assay

a. DPPH (1–1-diphenyl–2–picryl hydrazine) Free Radical Scavenging Assay

An examination of the total antioxidant activity of the methanol extract of *Alternantherabrasiliana* was determined by the DPPH method is indicated in Table 5. The standard for assay is ascorbic acid. Through probit analysis, IC50 value of the standard was found to be 48.6µg/mL and for the sample it was 52µg/mL.

Table 5. DPPH Assay

Sl. No.	Concentration (µg/mL)	Standard (percentage of inhibition) (%)	Plant extract (percentage of inhibition) (%)
1	62.5	68	64.65%
2	125	70	67.53%
3	250	73	69.81%
4	500	80	78.68%
5	1000	89	85.95%

b. Hydrogen Peroxide Scavenging Assay

The hydrogen peroxide scavenging activity of *Alternanthera brasiliana's* methanol extract was conducted. Ascorbic acid served as the standard for hydrogen peroxide assay. The extract's IC_{50} value was 73.166µg/mL and that of standard was 61.8µg/mL calculated by probit analysis. The sample shows good scavenging activity to hydrogen peroxide indicating potential antioxidant activity.

Table 6. Hydrogen peroxide assay

Sl. No.	Concentration (µg/mL)	Standard (percentage of inhibition %)	Plant extract (percentage of inhibition %)
1	62.5	52	47.81
2	125	61	57.17
3	250	65	63.04
4	500	75	69.31
5	1000	86	79.37

Statistical Analysis

Regression analysis was carried out for the methanol extract of *A. brasiliana* and Ascorbic acid (Standard) and the values were recorded (Table 7)The results of R^2 values (>0.1) showed statistical significance and indicated an adequate goodness of fit.

Table 7. Regression analysis in *Alternanthera brasiliana*

Assays	Regression values (R^2)		Regression equation	
	Plant	Standard	Plant	Standard
DPPH Assay	0.9342	0.989	7.7545ln (x) + 30.508	0.0224x + 67.333
Hydrogen peroxide scavenging assay	0.9903	0.9295	10.858ln (x)+ 33.895	0.0331x+ 54.958

5. HPTLC Analysis A(High Performance Thin Layer Chromatography)

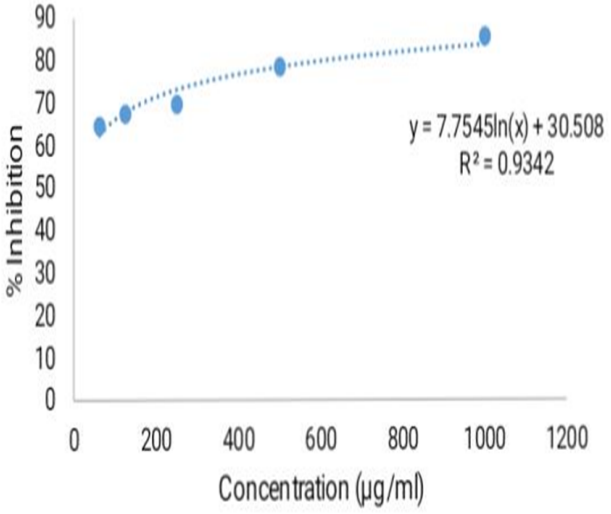
HPTLC analysis of hydromethanolic extract of *Alternanthera brasiliana* was done with the solvent system Toluene: ethyl acetate: methanol in the ratio 8: 3:1. Different Rf values were observed at three different wavelengths indicating the presence of different phytocompounds **(**Fig 3 , Graph 3,4,5**).**

Solvent system: Toluene: Ethyl acetate: Formic acid (5:3:0.1)

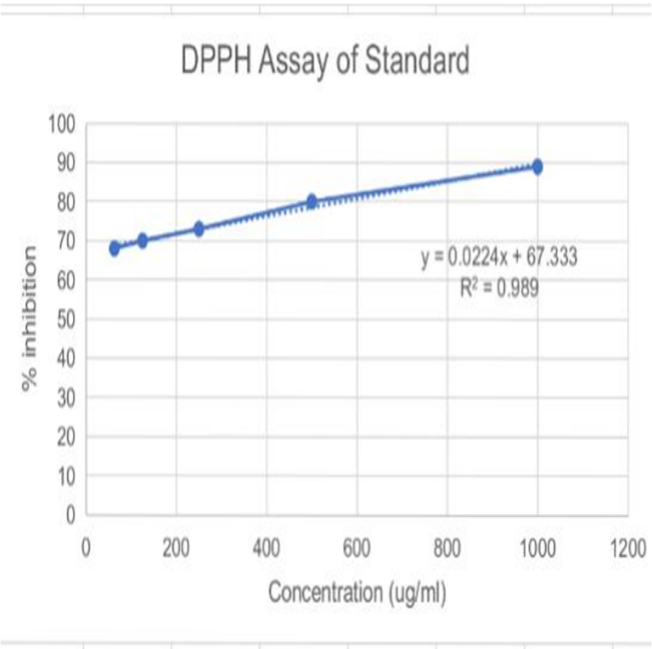
Track 1- 5µl, Track 2- 7µl

Graph – 1 DPPH assay

DPPH activity of sample

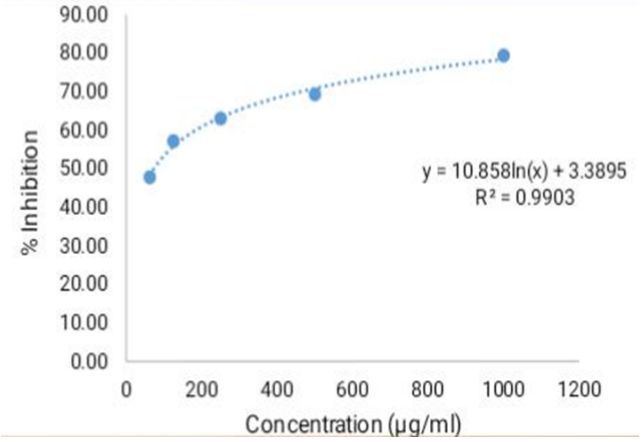


DPPH activity of standard

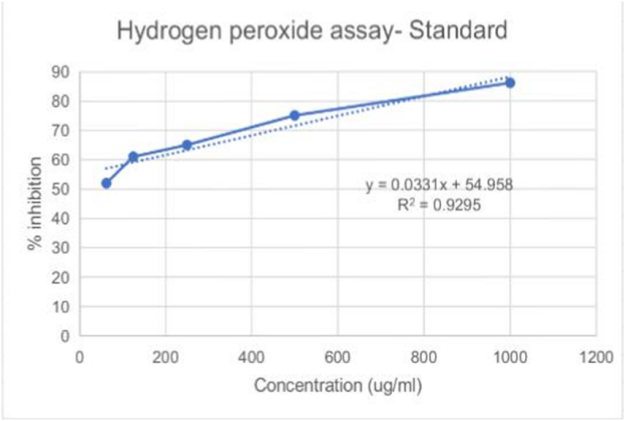


Graph 2 – Hydrogen peroxide assay

Hydrogen peroxide assay of sample



Hydrogen peroxide assay of standard



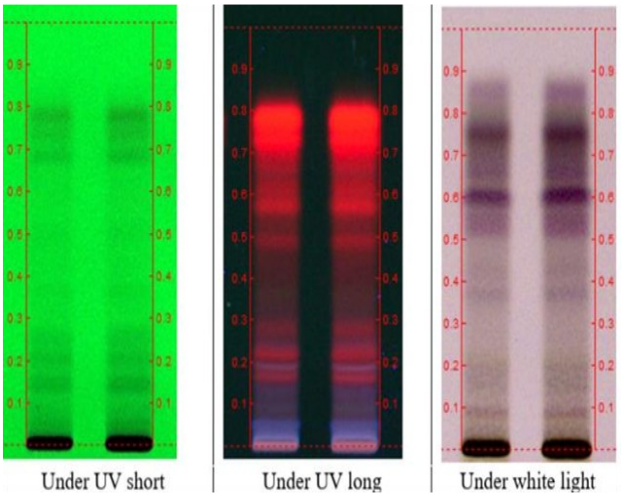


Figure 3. HPTLC test for *Alternanthera brasiliana*

Discussion

Alternanthera brasiliana is a variegated green and yellow, or bronze and green, or red and pinkish brown, perennial plant (Kumar et al., 2011) belonging to the family of Amaranthaceae. In traditional medicine, the plant has been reported to be good fodder which increases milk in cattle, and used in the treatment of cough, diarrhea (Brochado et al., 2003), decreases blood viscosity and reduces hypertension (Christian et al.,2006). Pharmacological studies of the leaves of the plant show that it has anti-oxidant, anti-microbial, anti-viral, anti-inflammatory and anti-nociceptive properties (Kumar et al., 2011; Barua et al., 2012) which could be attributed to the presence of flavonoids and phenols in the plant (Attaugwu and Uvere, 2017).

The analysis of medicinal plants has a long history in assessing the quality of a plant and the first analyses performed were organoleptic (Fitzgerald et al., 2020). Organoleptic is defined as being perceivable by the senses such as smell, appearance, taste, touch, odor etc. (Arya and Raneev, 2012). There are several ways in which to test the organoleptic properties of dried samples, including chemical or microscopic testing as well as by perceiving it directly through the senses. Organoleptic evaluation can be done by means of organs of sense and thereby define some specific characteristics

of the material which can be considered as a first step towards establishment of identity and degree of purity (Jarald and Jarald, 2007). The organoleptic study was conducted using *Alternanthera brasiliana* powder. It has an olive green colour, pungent smell, a bitter taste and a rough texture (Table 1).

The physicochemical standards are important to check the quality, purity and adulteration of given crude drug. The physiochemical characteristics were determined in *Alternanthera* (Table 2). Foreign organic matter is matter or part of the matter other than the crude drug which is not defined and described in the prescribed monograph of sample is known as foreign organic matter. High percentage of foreign organic matter is considered as a more deteriorating quality of drug or sample (Mukherjee ,2002). In the present study, small amount of foreign matter could be observed in *Alternanthera brasiliana*.

Deterioration time of the drug depends upon the amount of water present in the formulation and presence of excess moisture in the plant acts as an adulterant. If the water content is high, the formulation can be easily deteriorated due to fungal colonies. The moisture content was determined with reference to air-dried sample by loss on drying method which detects the net weight of a substance after drying at a specified temperature. It is an inevitable component of crude drug which helps in its preservation. The moisture content of this species in the present study was low (<14%) indicating that it could discourage bacterial, fungal or yeast growth (Raad,2014). A drug containing excess water leads to the activation of enzymes and will lead to the proliferation of living organisms. Although the loss in weight, in the sample so tested, principally is due to water, small amounts of other volatile materials will also contribute to the weight loss.

The medicinal plant materials contain saponins that cause the persistent foam formation when an aqueous decotion is shaken. The foaming ability of plant material and their extract is measured in terms of foaming index (Abdul *et al.*,2013). The swelling index was calculated to know that how much plant material can swell after putting in water and also to know that the plant material contains some mucilaginous content (Ahemed *et al.*,2009).Swelling index was zero, due to the lack of gums and mucilage in *Alternanthera brasiliana*. In the case of *Alternanthera brasiliana* the forming index was greater than 100 units. This indicates the presence of saponin glycosides in *Alternanthera brasiliana*. The pH is really a measure of relative amount of free hydrogen and hydroxyl ions in the water. Here the pH is 7.5, which indicates slightly alkaline nature of the drug powder.

Phytochemical screening is the most popular method of isolating medicinal principles. Studying, researching, extracting, and experimenting to identify various classes of phytoconstituents is a scientific process. It is a scientific method for examining, extracting, experimenting, and identifying diverse types of phytoconstituents present in various plant sections (Sharma et al., 2020). The existence of bioactive substances produced by plants, such as flavonoids, alkaloids, tannins, terpenoids, saponins, glycosides, phenolic compounds, *etc.*, is confirmed by phytochemical screening (Shad *et al.*, 2014).

Alternanthera brasiliana extract is used for phytochemical screening. The whole plant is used for extraction. The methanol extract of the plant was performed using the Soxhlet extraction method. Extraction methods used pharmaceutically involves the separation of medicinally active portions of plant tissues from the inactive/inert components by using selective solvents. The factors affecting the choice of the solvent are quantity of phytochemicals to be extracted, rate of extraction, diversity of different compounds extracted, diversity of inhibitory compounds extracted, ease of subsequent handling of the extracts, toxicity of the solvent

in the bioassay process and potential health hazards of the extractants (Antro et al.,2013). Since the end product will contain traces of the residual solvent, the solvent should be non-toxic and should not interfere with the bioassay (Tiwari et al., 2011). This technique produced a yield of 4.6%.

The presence/ absence of specific compounds is determined by specific colored products produced in reactions on the addition of specific chemicals. The procedure is simple and a preliminary pre-requisite before going for a detailed phytochemical investigation. Various pharmacological activities are expressed by medicinal plants based on the type and amount of biologically active secondary metabolites such as terpenoids, flavonoids, alkaloids and glycosides (Jegede et al., 2011).

Alternanthera brasiliana's methanolic extract was used to conduct preliminary phytochemical screening. The phytochemicals found in the extract included tannins, saponins, flavonoids, alkaloids, phlobatannins, simple phenolics, coumarins, quinones, flavanols, and lignin (Table 3). Quantitative estimation was also done for flavonoids (5.62 mg/g) and alkaloids (1.48 mg/g) in *Alternanthera brasiliana* (Table 4).

DPPH assay is relatively rapid and efficient method to evaluate free radical scavenging activity. DPPH is able to accept an electron or hydrogen radical to form a stable diamagnetic molecule. Changes in color, from purple to yellow indicates a decrease in absorbance of DPPH radical. This demonstrates that the antioxidant found in a mixture solution interact with the free radicals (S. B. Kedare and R. P. Singh,2011). DPPH assay was done in *Alternanthera brasiliana* for determining the antioxidant activity (Table 5). The methanol extract was taken for the assay. Ascorbic acid was used as the standard. The IC_{50} value of the plant extract was 52µg/ml by probit analysis comparable to standard. Thus the methanol extract of *Alternanthera brasiliana* exhibited good antioxidant activity (Table 5,Graph 1).

The hydrogen peroxide involved the scavenging potential of the extract on the free radicals of peroxide. The ability of any compound or substance to be able to mop up H2O2 in any chemical reaction is a proof that such a substance will have protective activity against the hydroxyl radicals being generated by hydrogen peroxide. Hydrogen radicals have been reported to be engendering cytotoxicity in living tissues (Al-Owaisi et al., 2014). The hydrogen peroxide assay in the present study of *Alternanthera brasiliana* revealed that it had high scavenging activity towards hydrogen peroxide by having a IC_{50} value of 73.16ug/ml comparable to standard (Table 7, Graph 2)

The IC_{50} values obtained by probit analysis revealed that the methanol extract of *A.brasiliana* possessed good antioxidant activity comparable to the drug ascorbic acid. The results were statistically significant (>0.1) on regression analysis conducted in the plant and standard and indicated an adequate goodness of fit (Table.7)

HPTLC (high-performance thin layer chromatography) is an advanced type of TLC that gives high resolution and accurate results. The Rf values calculated for the phytoconstituents present in the tested sample would be helpful in the identification of the unknown compounds by comparing them with the reference standards, and from the values of peak area, the concentration of the compounds can be determined. The bands of separated compounds can be seen (Figure 3) on the TLC plates visualized under UV short, UV long and white light of wavelengths 254 nm, 366 nm and 575nm respectively.

Alternanthera brasiliana clearly indicates the potential utilization of the plant singly or in certain formulation to treat several disease or disorders of humans. The plant can be grown in available area for medicinal purposes. Nevertheless, based on the above presented results, the plant could be investigated as a possible

new source of natural antioxidants in the food, nutraceuticals and cosmetic industry.

Conclusion

The present study on pharmacognostical evaluation of *Alternanthera brasiliana* will provide useful information for its identification and these parameters can used in future to assess the quality and purity of *Alternanthera brasiliana*. The phytochemical parameters and analytical methods will provide useful information in the field of crude drug development. The results of the present study provided suitable standards for identification of this medicinally important plant drug material for future investigations and applications. The *in vivo* studies should be followed by clinical trials in man. The data obtained in the present work will be useful in the synthesis of new drugs of pharmaceutical importance from *A. brasiliana.*

References

Abdul W. Mehreen G. Syed BJ and Muhammad N. (2013). Phytochemical analysis of medicinal plants occurring in local area of Mardan. Journal of Biochemistry and Analytical Biochemistry 2(4).

Ahmad I, Aqil F and Owais M (2006). Modern phytomedicine: Turing medicinal plants into drugs. Wiley – VCH Verlag GmbH & Co Weinheim.

Ahmed A, Alkarkhi AFM, Hena S. and Khim LH. (2009). International Journal of Chemistry 1(1): 36-49.

Anees A. Abbas FM. Sufia H and Lim HK. 2009. Extraction, separation and identification of chemical ingredients of Elephantopus scaber L. using factorial design of experiment. International Journal of Chemistry 1(1).

Dar RA, Shahnawaz M and Qazi PH. 2017. General overview of medicinal plants: A review. The Journal of Phytopharmacology 6(6): 349-351.

Day A. Dupont MS, Ridley S. Rhodes MJ and Morgan MR. 1998. Deglycosylation of flavonoid and isoflavonoid glycosides by human small intestine and liver beta-glucosidase activity. FEBS Lett. 436, 71-75.

Deepak MK, Surendra SK and Hanhong B. 2015. Significance of antioxidant potential of plants and its televance to therapeutic applications. International Journal of Biological Sciences 11(8): 982-991.

Indian Pharmacopoeia. 1992. Volume II. Part I. The Controller of Publications, CSIR, New Delhi.

Jarald EE, Jarald SE. A text book of pharmacognosy and phytochemistry. 1st edition, CBS Publishers and distributors, New Delhi, India, 2007; 6.

Jegede, I. A., Ibrahim, J. A and Kunle, O. F. 2011. Phytochemical and pharmacognostic studies of the leaf and stem bark of Anthocleista vogelii (Planch). Journal of Medicinal Plants Research, 5(26): 6136-6139.

Males Z, Plazibat M, Bilusiae V, Vundae ZI, Hazler KP (2004). Thin-layer chromatographic analysis of flavonoids, phenolic acids, and amino acids in some Croatian hypericum Taxa. J. Planar. Chromatogr.;17:280–285.

Maroyi, A., Cheikhyoussef, A., (2015). A comparative study of medicinal plants used in rural areas of Namibia and Zimbabwe. Indian J. Tradit. Knowl.

Medic-Saric M, Jasprica I, Mornar A, Males Z (2008). Thin Layer Chromatography in Phytochemistry. 2nd ed. CRC, Press;. Application of TLC in the isolation and analysis of flavonoids.

Pandey K.B., Rizvi S.I (2009). Plant polyphenols as dietary antioxidants in human health and disease. Oxid. Med. Cell Longev.;2:270–278. doi: 10.4161/ oxim.2.5.9498.

Paramita V., Kusumayanti H., Amalia R., Leviana W., Nisa Q.A (2018). Application of Flavonoid and Anthocyanin Contents from Rambutan (Nephelium lappaceum) Peel as Natural Dyes on Cotton Fabric. Adv. Sci. Lett.;24:9853–9855. doi: 10.1166/asl.2018.13160.

Phongpaichit, S. J. Nikom, N. Rungjindamai, J. Sakayaroj, N. Hutadilok-Towatana, V. Rukachaisirikul and K. Kirtikara ((2007).), FEMS Immunol. Med. Microbiol.

51 (3), 517-525

Popovic Z., Matic R., Bojovic S., Stefanovic M (2001–2013)., Vidakovic V. Ethnobotany and herbal medicine in modern complementary and alternative medicine: An overview of publications in the field of I&C medicine. J. Ethnopharmacol. 2016;181:182–192. doi: 10.1016/ j.jep.2016.01.034.

Pratap CR (2017). Analysis of proximate, phytochemical, elemental compositions and antioxidant property of leaf of Alternanthera brasiliana (l.) kuntze. MOJ Food Process Technol. 4(3):74-79.

Raad AK. 2014. Physico-chemical parameters, phytochemical screening and antioxidant activity of seeds of Peganum harmala collected from Iraq. Asian Journal of Biomedical and Pharmaceutical Sciences 4(28): 20-24.

Sharma, T., Pandey, B., Shrestha, B. K., Koju, G. M., Thusa, R., & Karki, N. (2020). Phytochemical Screening of Medicinal Plants and Study of the Effect of Phytoconstituents in Seed Germination. Tribhuvan University Journal, 35(2), 1–11.

Shkondrov A., Krasteva I., Pavlova D., Zdraveva P. Determination of flavonoids in related Astragalus species (Sect. Incani) occurring in Bulgaria. Comptes rendus de l'Académie Bulg. des Sci. 2017;70:363–366.

Sisodiya D, Shrivastava P (2017) Qualitative and quantitative estimation ofbioactive compounds of Euphorbia thymifolia L. Asian J Pharm Edu Res 6(3):34–4310.

Wagner H, Bladt S (2001). Plant Drug Analysis. A Thin

Layer Chromatography Atlas. Berlin : Springer;.

Xiao Y, Zhang S, Tong H, Shi S (2018). Comprehensive evaluation of the role of soy and isoflavone supplementation in humans and animals over the past two decades. Phytother Res. 32:384–94. 10.1002/ptr.5966

YInger, H. & Yewhalaw, D. (2007). Traditional medicinal plant knowledge and use by local healer in Sekora district, Jimmazone, South-Eastern Ethiopia. Journal of Ethnobiology and Ethnomedicinal, 3:24-30.