Bmed-H
e-ISSN: 2582-7405
Transaction on Biomedical Engineering Applications and Healthcare
Vol-2 Issue-1, April 2021

Phytochemical Analysis and Biological Activities of *Alternanthera Braseliana* Kuntz Leaves and Stem Extracts

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Abstract

Alternanthera brasiliana Kuntz is an important herb which is having higher medicinal properties. Through all most all of the parts are used in the traditional system of medicines. The bioactive compounds or phytochemicals present in the plant show many pharmacological activities like wound healing, anti-inflammatory, antitumor, analgesic, immunostimulant and, antimicrobial and antiviral activities. Phytochemical screening of methanolic and ethanolic extracts of, A.brasiliana leaves showed the presence of phenol and alkaloids and A.brasiliana stem extracts showed the presence of different constituents like alkaloids flavonoids steroids glycosides and saponin. The DPPH and ABTS methods were followed for studying antioxidant activities of extracts. The wound healing potential of extracts is determined by the in-vitro method by using chick embryo fibroblast cell line study revealed that both stem and leaf showing wound healing activity. From the extracts the ethanolic extracts showing great wound healing potential at a time interval. Ethanolic stem extracts show comparatively higher wound healing potency. This paves way for future studies on this plant for isolation and commercialization of phytochemicals which are responsible for wound healing property of the plant Alternanthera brasiliana Kuntz.

Index Terms

Alternanthera brasiliana, antioxidant, DPPH and ABTS, wound healing, MTT assay

INTRODUCTION

The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents [1]. The use of medicinal plants by people in developing countries is popular because these products are safe, widely valuable at low cost and easy to access [2]. An estimated 70,000 plants (including lower plants) are used medicinally. Out of 17,500 flowering plants known to occur in India, about 3000 are recognized for their medicinal use and they are placed in three categories, which are plants of codified knowledge used in system of medicine like Ayurveda, unani and siddha, plants of empirical knowledge used in folk medicine and plant of scientific knowledge [3].

Alternanthera brasiliana Kuntz, belong to family *Amaranthaceae* is an herb widely used by rural communities as medicinal agent to cure different disease, such as inflammation, and dolorous or infection processes, wound healing, analgesic, antitumor activity, immunomodulator and lymphocyte proliferation. *A.brasiliana* produce compound with possible analgesic action with influence of different kind of lights[4]. The elements, like P, S, K, Ca, Mn, Fe, Cu, Zn, Sr, and Pb were detected in all samples, and the elements Cl, Ti, Cr, Co, Ni, Br, Rb, Sr, Cd, Sn, Sb, and Ba were detected in some samples of *A. brasiliana* [5].

Phytochemical screening of the extract of the plant revealed the presence of different classes of secondary metabolites such as alkaloid, steroid, and triterpenes [6].

Wound healing is a complex phenomenon that results in restoration of anatomic continuity and function, accomplished by several processes which involve different phases including inflammation, granulation, fibrogenesis, neo-vascularization, wound contraction, and epithelization [7]. Wounds can be broadly classified into acute and chronic wounds depending on their etiology, acute wounds occur most commonly due to accidents such as trauma or burns. Acute wounds should normally heal in a short duration provided the right treatment is given [8]. The earliest known record of the treatment of wounds was found on clay tablets of Mesopotamian origin from about 2500 BCE [9]. Mesopotamians washed wounds with milk or water before dressing with honey or resin [10]. Recent trends indicate that materials used to maximize wound healing in the future will utilize a wide range of nanotechnologies as smart dressings that respond to the wound environment, with dressings capable of releasing biomolecules or producing a signal currently being developed [11].

The aim of the present study is obtaining maximum extraction from stem and leaves of Alternanthera brasiliana Kuntz, identification of phytoconstituents present in each extracts and the wound healing potential of extracts. The wound healing efficiency studies of *Alternanthera brasiliana* is carried out mostly in leaf extracts. The present study investigates the wound healing efficiency of *Alternanthera brasiliana* with both leaf and stem extracts. However, this is the first wound healing efficiency study with stem extracts of *Alternanthera brasiliana* in fibroblast cell culture.

MATERIALS AND METHODS

Sample collection and preparation of plant material

The leaf and stem of *Alternanthera Braseliana* were collected during the flowering season (December 2016) Kannadiparamba in Kannur district, Kerala-India taxonomically identified at the Botanical Survey of India (BSI) (Accession Number 2116). Fresh leaves and stem of the plant were cleaned from extraneous materials, washed and air-dried in shade. Dried plant materials are powdered mechanically, weighed, and stored in airtight containers. The extraction process was carried out with two pure solvents of varying polarities methanol and ethanol. Extraction of the air-dried, powdered leaves (60.0g) was done (maceration at room temperature) with ethanol and methanol separately at a 1:1 (w/v) ratio of plant material powder mass/solvent in the rotary shaker. The powdered stem (40.0g) also extracted at a 1:0.8 (w/v) ratio respectively. The extract was obtained after 48 hours through filtration method with the help of Whatman no. 1 filter paper thrice and the solvent was removed by evaporation at 400C. The final dark residue of the extract is stored in an airtight container until use.

Phytochemical analysis [12] [13]

Methanol and ethanol extracts of Alternanthera brasiliana were subjected to various phytochemical analyses using standard methods.

Alkaloids

The extract was mixed with 2 ml of Dargendroff's reagent and the prominent orange or yellow precipitate indicated the presence of alkaloids. Similarly, with one drop of Mayer's reagent, the formation of white or creamy precipitate indicated the presence of alkaloids.

Flavonoids [14]

The extract was treated with 5% NaOH and 10% HCl solution and the yellow solution turns colourless indicated the presence of flavonoids. Similarly, with few drops of sodium hydroxide solution, the formation of intense yellow colour, which becomes colourless on the addition of dilute acid, indicates the presence of flavonoids.

Phenols

The presence of Phenols was detected using Ferric chloride. 1ml of the extract was treated with a few drops of neutral 5% ferric chloride solution. Dark green colour indicates the presence of phenol compounds.

Phytosterols

The presence of Steroids was detected using Libermann-Burchard's test where 1 ml of acetic anhydride was added to 1ml of extract each and followed by 1ml of H2SO4. The colour change from violet to blue or green of samples indicates the presence of steroids**.**

Terpenoids

The presence of terpenoids was detected using Libermann-Burchard's test where 1 ml of extract was treated with 1 ml of acetic anhydride, boiled and cooled. Then 1 ml concentrated sulphuric acid is added by the side of the test tube, brown rings formed at the junction two layers and the upper layer turned greenish which shows the presence of steroids and formation of deep red colour indicates the presence of Triterpenoids. Similarly, using Salkowski's test, 1 ml of extract was treated with 1ml of chloroform and filtered. The filtrates were treated with few drops of concentrated sulphuric acid, shaken and allowed to stand. The appearance of the golden yellow colour indicates the presence of triterpenoids.

Saponins

The presence of saponins was detected using a foam test where 1ml of the extract is diluted with the same amount of distilled water. The suspension is shaken method and the formation of foam indicates the presence of saponins.

Glycosides

Glycosides detection was tested using the Keller-Kiliani test in which 2ml of the extract was mixed with 1ml glacial acetic acid, three drops of Iron (III) chloride, and concentrated sulphuric acid. Green-blue colour indicates the presence of cardiac glycosides.

Fixed oils and fats

The detection was made with a spot test by pressing a small quantity of extract between two filter papers and the oil stain indicates the presence of fixed oil. A pink or violet colour in the base layer indicates the presence of anthraquinones when 1ml of the extract was treated with the same volume of aqueous base NaOH or NH4OH solutions.

Proteins

The extract was treated with I ml of 40% sodium hydroxide solution and two drops of 1% copper sulphate reagent. The appearance of violet colour indicates the presence of proteins. Likewise, to 1 ml of extract, two drops of freshly prepared 0.2% Ninhydrin reagent was added and heated. Development of purple colour indicates the presence of proteins.

Carbohydrates

The presence of carbohydrates was detected using Fehling's and Benedict's test. The extract was treated with 5ml of Fehling's solution (A and B) and kept in boiling water bath for 5 minutes. Formation of a yellow or red colour precipitate indicates the presence of reducing sugar. To 1ml of the extract, added 5ml of Benedict's solution and kept at boiling water bath for 5 minutes. Red, yellow or green precipitate indicates the presence of reducing sugars.

FTIR (Fourier-Transform Infrared Spectroscopy) analysis [14]

Fourier transform infrared spectrophotometer (FTIR) is perhaps the most powerful tools for identifying the types of chemical bonds (functional groups) present in compounds. Dried powders of different solvent extracts of each plant

material were used for FTIR analysis. 10mg of the dried extract powder was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample disc. The powdered sample of each plant specimen was loaded in FTIR Spectroscopy (Shimadzu, IR Affinity1, Japan), with a scan range from 400 to 4000 cm-1 with a resolution of 4cm-1.

GC-MS analysis [15] [16]

The Clarus 680 GC was used in the analysis, employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250µm df) and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min. The injector temperature was set at 260°C during the chromatographic run. The 1µL of each extracted sample injected into the instrument. The oven temperature was as follows: 60 °C (2 min); followed by 300 °C at the rate of 10 °C min−1; and 300 °C, where it was held for 6 min. The mass detector conditions were: transfer line temperature 240 °C; ion source temperature 240 °C; and ionization mode electron impact at 70 eV, scan time 0.2 seconds and scan interval of 0.1 seconds. The fragments from 40 to 600 Da. The spectrums of the components were compared with the database of the spectrum of known components stored in the GC-MS NIST (2008) library.

DPPH Radical Scavenging Assay [17]

The DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical is widely used as the model system to investigate the scavenging activities of several natural compounds. DPPH radical is scavenged by antioxidants through the donation of proton forming the reduced DPPH. Radical scavenging activity increased with an increasing percentage of free radical inhibition. Antioxidants react with DPPH, which is a stable free radical and is reduced to the DPPH-H and as a consequence, the absorbance's decreased from the DPPH radical to the DPPH-H form. The colour changes from purple to yellow after reduction, which can be quantified by its decrease of absorbance at wavelength 517 nm. The degree of discolouration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability. About 0.1 mM solution of DPPH (1, 1-Dipheny- 2-Picrylhydrazyl) in ethanol was prepared and 1.0 ml of this solution was added to 3.0 ml of extract solution in water at different concentrations (20-100 mg /ml). Thirty minutes later, the absorbance was measured at 517 nm. A blank was prepared without adding extract. Ascorbic acid at various concentrations (20 to 100 mg/ml) was used as standard. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation:

DPPH Scavenged (%) = [(A control – A test) / A control] ×100

Where A control is the absorbance of the control reaction and A test is the absorbance in the presence of the sample of the extracts. The antioxidant activity of the extract was expressed as IC50 and compared with standard. The IC50 value was defined as the concentration (in mg/ml) of extracts that inhibits the formation of DPPH radicals by 50 [18].

ABTS radical scavenging method [18 – 21]

The pre-formed radical monocation of 2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ABTS is generated by oxidation of ABTS with potassium persulfate (a blue chromogen) and is reduced in the presence of such hydrogen donating antioxidants. Weigh 15 mg of extracts and the standards (ascorbic acid) and dissolve in 2mL of DMSO. Then serially dilute with DMSO to obtain the lower dilutions. To 0.2mL of various concentrations of the extracts or standard, add 1mL of distilled DMSO and 0.16mL of ABTS solution make a final volume of 1.36 mL Measure absorbance, after 20min at 734nm using ELISA reader. Calculate the IC50 value and compare with standard IC50 value.

MTT assay [22-23]

MTT Cell Proliferation and Viability Assay is a safe, sensitive, in vitro assay for the measurement of cell proliferation or, when metabolic events lead to apoptosis or necrosis, a reduction in cell viability. Fibroblasts Cells are isolated from 15-day old chick embryo and cultured in flat-bottomed, 24-well tissue culture plates. Ethanol and Methanol extracts of leaf and stem of *A.brasiliana* were added at a concentration of (6, 12, 25, 55, and 85 mg). The cells are treated as per the experimental design and incubation times are optimized for each cell type and system. The tetrazolium compound MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) is added to the wells and the cells are incubated. MTT is reduced by metabolically active cells to insoluble purple formazan dye crystals. Detergent is then added to the wells, solubilizing the crystals so the absorbance can be read using a spectrophotometer. Samples are read directly in the wells. The optimal wavelength for absorbance is 570 nm, but any filter that absorbs between 550 and 600 nm may be used. The data is analyzed by plotting cell number versus absorbance, allowing quantitation of changes in cell proliferation. The rate of tetrazolium reduction is proportional to the rate of cell proliferation.

Wound Healing Activity- Determination of LD50 [24-30]

Fibroblast Cells were isolated from 15 days old chick embryo and cultured in flat-bottomed, 24-well tissue culture plates. The cells were treated as per the experimental design and incubation times are optimized for each cell type and system. Then after 24 hours to 48 hours of incubation, a part of the fibroblast cells was rubbed out to make a wound in the cell line. Then the fibroblast cells were treated with colchicine to make senescence. After treated with colchicine the cells were treated for the wound healing activity by ethanolic and methanolic extracts of leaf and stem of *A.brasiliana* with different concentrations from 6, 12, 25, 55, and 85.

RESULTS

The stem and leaves samples were extracted by cold extraction method using ethanol and methanol. The amount of leaf extracted was 1930mg/60gm in ethanol and 2110mg/60gm in methanol similarly the amount of stem extraction was found to be 1714mg/60gm in ethanol and 1840mg/60gm in methanol. From these results the methanol solvent given maximum amount of leaf extract obtained as 2110 mg/60 gm and the remaining solvent extract obtain lower than the leaf methanol, like wise methanol solvent given maximum amount of stem extract obtained as 1840 mg/60gm.

Phytochemical Analysis- Qualitative

The phytochemical analysis in both leaf and stem extracts of methanolic and ethanolic extracts of *Alternanthera brasiliana* showed the presence and absence of phytochemical constituents as reported in Table 1. Both leaf and stem extracts showed the presence of alkaloids, except ethanolic extracts of stem all other extracts showed presence of phenols. Flavonoids are present in both ethanolic and methanolic extracts of stem. Presence of glycosides only showed in ethanolic stem extract.

Table.1. Phytochemical screening of methanolic and ethanolic leaf and stem extracts of *Alternanthera brasiliana*

Phytochemical constituent	*Test*	Methanolic leaf Extracts	Ethanolic leaf Extracts	Methanolic stem extracts	Ethanolic stem extract
Alkaloids	Dragendorff‟s	+	+	+	+
	Mayer „s Test	+	+	+	+
Flavanoids	10% HCL, 5% NaOH	–	+	+	+
	Alkaline Reagent	–	–	-	-
Phenols	Ferric Chloride	+	+	+	-
Phytosterols	Libermann-Burchard‟s	–	–	-	-
Terpanoids	Libermann-Burchard‟s	–	–	-	-
	Salkowski‟s	–	–	-	-
Saponins	Foam Test	–	–	+	+
Glycosides	Keller – Kiliani	–	–	-	+
Fixed oils and Fats	Spot Test	–	–	-	-
	Anthraquinone	–	–	-	-
Amino acids and Priteins	Ninhydrin	–	–	-	-
	$CuSO_4$ & NaOH	–	–	-	-
Carbohydrates	Fehling‟s Test	–	–	-	-
	Bendict‟s Test	–	–	-	-

Present (+) Absent(–)

FTIR Spectral Data Interpretation

The FTIR spectrum of leaf and stem extracts (prepared in two different solvents) of *alternanthera brasiliana* are given in Fig.3-6. The data on the peak values and the probable functional groups (obtained by FTIR analysis) present in the leaf and stem extracts of *A.brasiliana* are presented in Table.2-5.

The methanol extracts of leaf of *A.brasiliana* exhibit characteristic bands at 2956 cm^{-1}, 2924.09 cm^{-1} and 2852.72 cm^{-1} for carboxylic (O-H) and alkanes (C-H).The peak values showing the presence of compounds with the functional groups like phenols, carboxylic acids, Amines, amides, aromatic amines, aldehydes, alkanes and alkenes. The ethanolic extracts of leaf of *A.brasiliana* showed characteristic absorption bands at 3356.14 cm^{-1} and 2922.16 cm^{-1} for carboxylic (O-H) and alkanes (C-H) groups, 2852.72 cm^{-1} and 1625.99 cm^{-1} for carbonyl (C=O) and primary amine (C-H bending) respectively. Excluding these peak others adsorption band showed the presence of aromatic amines, esters, alkyl halides, aldehydes, alkenes and 1°, 2° amines.

The methanolic extracts of stem of *A.brasiliana* showed the characteristic absorption bands at 3292.49 cm^{-1} for amines and amides (N-H stretch) groups, 2927.94 cm^{-1} and 2854.65 cm^{-1} for carboxylic (O-H) and alkanes (C-H) groups and at 1336.67 cm^{-1} for nitro compounds (N-O). Different peaks showing the presence of compounds with different functional groups which includes alkenes, aliphatic amine, alcohols, aldehydes, carbonyls and esters. The ethanolic extracts of stem exhibit characteristics bands at different peaks from the minimal value 866.04 $cm^{-1}$for amines (N-H) and aromatic (C-H) groups to maximal value 3305.99 cm^{-1} for alcohol (O-H stretch) and phenol (H bonded). The characteristic bands were exhibited and forms different peaks according to their adsorption which showed the presence of function groups like alkene, esters, ethers, alcohols, carboxylic, alkynes and amine.

The FTIR data of both stem and leaf extracts (prepared each in methanol and ethanol) of *A.brasiliana* reveals the presence of characteristic functional group including

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aldehydes, carboxylic acids, esters, amines, alkenes, alkanes and carboxylic acids which may responsible for various medical properties of *Alternanthera brasiliana* including wound healing property.

Fig.3. FTIR spectrum of ethanolic extract of leaf of *A.brasiliana*

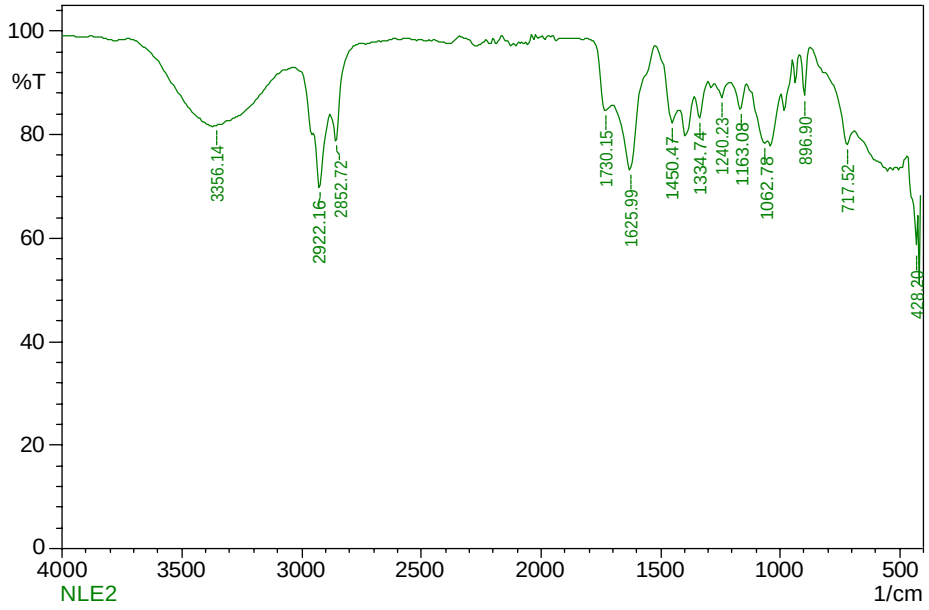

Table.2. FTIR spectral peak values and functional groups obtained for the leaf ethanol extract *A.brasiliana*

S.No	Peak Value (Cm-1)	Type of Bond	Functional Group
1	3356.14	O-H Stretch, H-Bonded, N-H Strctch	Phenols, Amines, Amides
2	2922.16	O-H Stretch	Carboxylic Acids, Alkanes
3	2852.72	O-H Stretch, C-H Stretch	Carboxylic Acids, Alkanes
4	1730.15	C=O Stretch	Carbonyls (General), Carboxylic Acids Aldehydes, Saturated Aliphatic
5	1625.99	C–H Bend	1° Amines
6	1450.47	C–H Bend, C–H Rock	Aromatic Alkanes Alkanes
7	1334.74	C–N Stretch, N–O Symmetric Stretch	Aromatic Amines, Nitro Compounds
8	1240.23	C-O Stretch, C-H Wag (–CH2X), C-N Sretch	Alcohols, Carboxylic Acids, Esters, Ethers, Alkyl Halides, Aliphatic Amines
9	1163.08	C-O Stretch C-H Wag (–CH2X)	Alcohols, Carboxylic Acids, Esters, Ethers, Alkyl Halide
10	1062.78	C-O Strtch, C-N Strtch	Alcohols, Carboxylic Acids, Esters, Ethers Aliphatic Amines
11	896.9	N-Hwag, =C-H C-Cl Stretch	1°, 2° Amines Alkenes Aromatics
12	717.52	N-Hwag =C-H Bend C"Oop", C-Cl	1°, 2° Amines, Aromatics, Alkyl Halides

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Fig.4. FTIR spectrum of methanolic extract of leaf of *A.brasiliana*

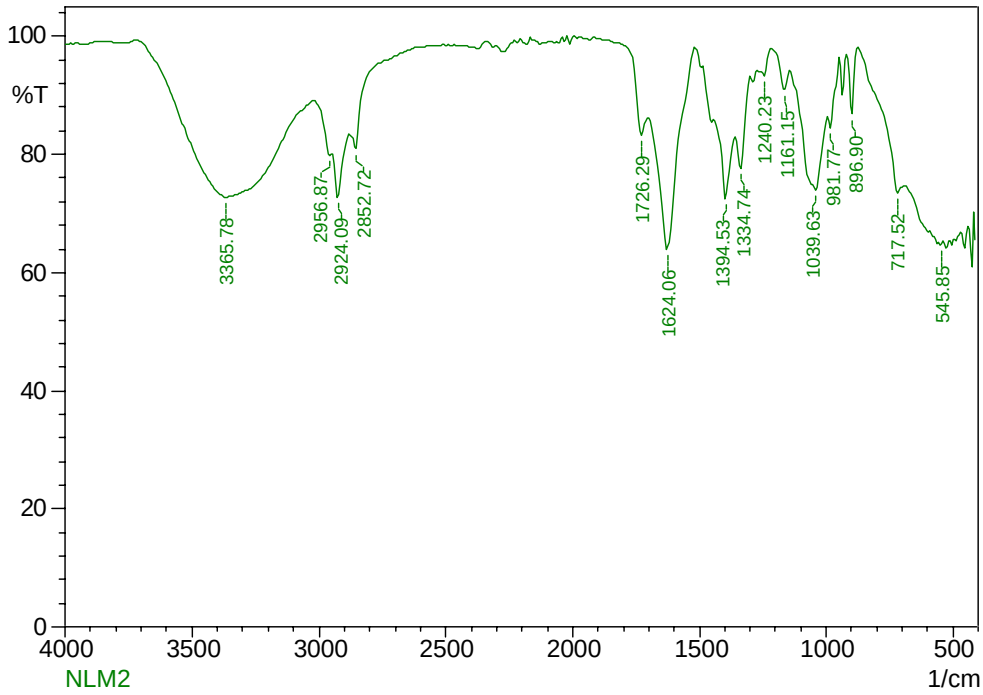

Table.3. FTIR spectral peak values and functional groups obtained for the Leaf Methanol Extract of *A.brasiliana*

S. NO	Peak value (cm-1)	Type of bond	Functional group
1	3365.78	O-H stretch, H bonded, N-H strctch	Alcohols, Phenols Amines, Amides
2	2956.87	O-H stretch, C-H stretch	Carboxylic Acids Alkanes
3	2924.09	O-H stretch	Carboxylic Acids
4	2852.72	O-H stretch,C-H stretch	Carboxylic Acids Alkanes
5	1726.29	C=O stretch	Carbonyls (General) Carboxylic Acids
6	1624.06	N-H bend	1° Amines
7	1334.74	C–N stretch, N–O symmetric stretch	Aromatic Amines, Nitro Compounds
8	1240.23	C-O stretch, C-H wag (–CH2X), C-N sretch	Alcohols, Esters, Ethers Alkyl Halides, Aliphatic Amines
9	1161.15	C-O stretch, C-H wag (–CH2X)	Alcohols, Carboxylic Acids, Esters, Ethers
10	1039.63	C-O strtch, C-N strtch	Alcohols, Carboxylic Acids, Esters, Ethers, Aliphatic Amines
11	981.77	N-Hwag, =C-H, C-Cl stretch	1°, 2° Amines, Alkenes Aromatics
12	869.9	=C-H bend, C-H "oop"	Alkenes, Aromatics

e-ISSN: 2582-7405

Fig.5 FTIR spectrum of methanolic extract of stem of *A.brasiliana*

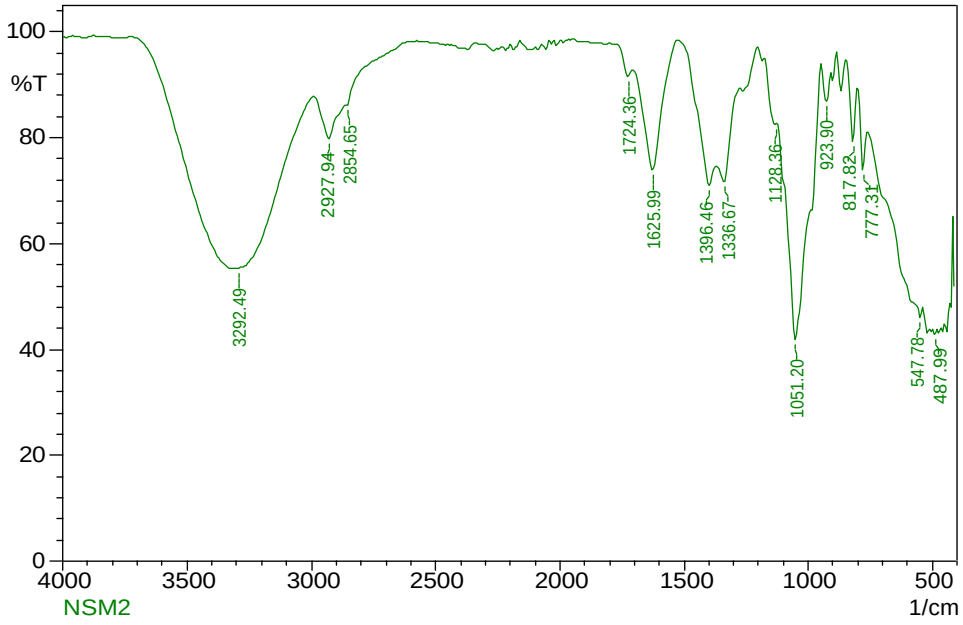

Table.4. FTIR spectral peak values and functional groups obtained for the Stem Methanol Extract of *A.brasiliana*

S.No	Peak Value (Cm-1)	Type Of Bond	Functional Group
1	3292.49	O–H stretch,H–bonded N–H stretch -C C–H: C–H stretch	alcohols, phenols amines, amides alkynes (terminal)
2	2927.94	C-H stretch,O-H stretch	Alkanes, carboxylic acids
3	2854.65	C-H stretch, O-H stretch	Alkanes, carboxylic acids
4	1724.36	C=O stretch	carboxylic acids, carbonyls aldehydes, saturated aliphatic
5	1625.99	N–H bend	1° amines
6	1336.67	N–O symmetric stretch	nitro compounds
7	1128.36	C–N stretch	aliphatic amines
8	1051.2	C–O stretch, C–N stretch	alcohols, carboxylic acids, esters, ethers, aliphatic amines
9	923.93	O-H bend, =C-H	Alkenes, carboxylic acids
10	817.82	N–H wag, C–H “oop”, C–Cl stretch	Alkenes, 1°, 2° amines, aromatic, alkyl halides
11	547.78	C–Br stretch	alkyl halides

Fig.6 FTIR spectrum of ethanolic extract of stem of *A.brasiliana*

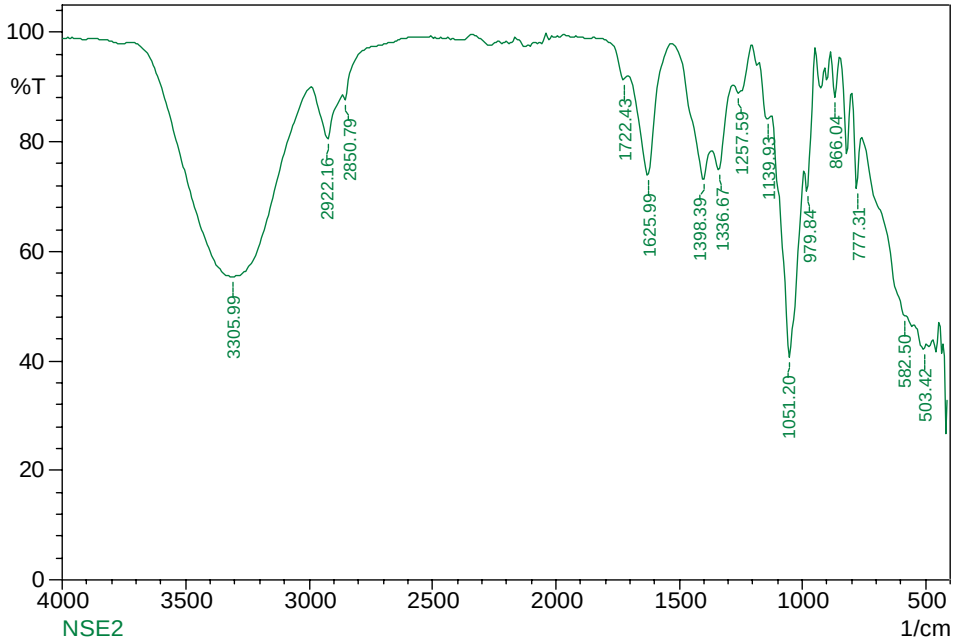

Table.5. FTIR spectral peak values and functional groups obtained for the Stem Ethanol Extract.

S. No	Peak Value (Cm- 1)	Type Of Bond	Functional Group
1	3305.99	O–H stretch, H–bonded,C–H: C–H stretch	alcohols,phenols, alkynes
2	2922.16	O–H stretch	carboxylic acids
3	2850.79	O–H stretch, C– H stretch	carboxylic acids,alkanes
4	1722.43	C=O stretch	carbonyls,esters, saturated aliphatic carboxylic acids, aldehydes
5	1625.99	N–H	1° amines
6	1336.67	N–O	nitro compounds
7	1257.59	C–N stretch	aromatic amines
8	1139.93	C–N stretch, C–O stretch	aliphatic amines alcohols, carboxylic acids, esters, ethers
9	1051.2	C–N stretch, C–O stretch	aliphatic amines, alcohols, carboxylic acids, esters,ethers
10	979.84	.=C–H	Alkenes
11	866.04	N–H wag, =C–H bend, C–H “oop”	1°, 2° amines,alkenes aromatic

GC-MS Anlaysis of stem and leaf extracts (methanolic and ethanolic) of *Alternathera brasiliana*

The GC-MS detected at least 30 constituents in each of the total injected plant parts extracts and the chromatogram were obtained as represented in fig.7 to 10. These constituent peaks were identified by comparing each mass spectrum with NIST and NISTREP mass spectra library of GC-MS data system. In the present study the GC-MS analysis of each extracts showing the presents more than 40 compounds. From these compounds some compounds showing higher area % from other compound. In leaf ethanolic extract 16-heptadecenal and 3,7,11,15, tetramethyl-2-hexaden-1-ol and their area % value are 22.25 and 18.058 respectively are in high amount. The compound 2r-Acetoxymethyl-1,3,3-Trimethyl-4t-(3-Methyl-2-Buten-1-Yl

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)-1t-Cycloh was identified in ethanol extract of leaf with area % 33.196. GC-MS analysis of methanol stem extract 9,12 octadedienoic acid(Z,Z) and tridecanoic acid are more area % and the compounds1,9 eicosadiene (61.169 are %) and N-hexadecanoic acid (32.656 area %) are identified in ethanolic extract of stem, These phytoconstituents showing different biological activities (Table.6 to 9) including cell adhesive property, anticancer activity and antimicrobial activity.

Fig.7 GC-MS analysis for methanolic leaf extract of the plant sample

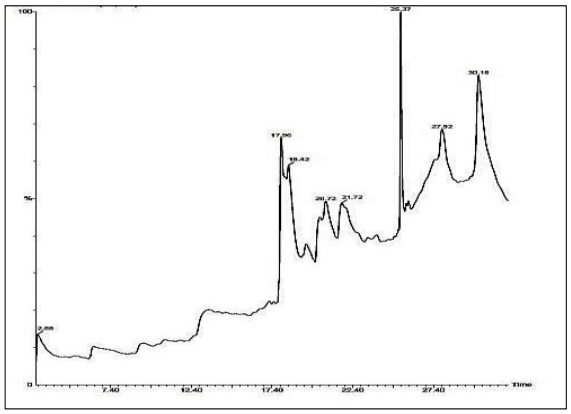

Fig.8 GC-MS analysis for ethanol leaf extract of the plant sample.

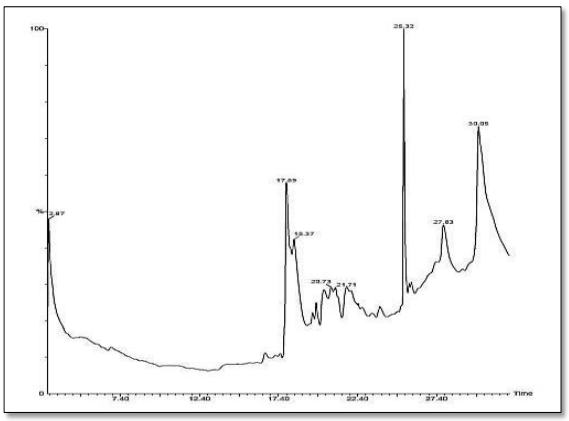

Fig.9 GC-MS analysis for methanol stem extract of the plant sample

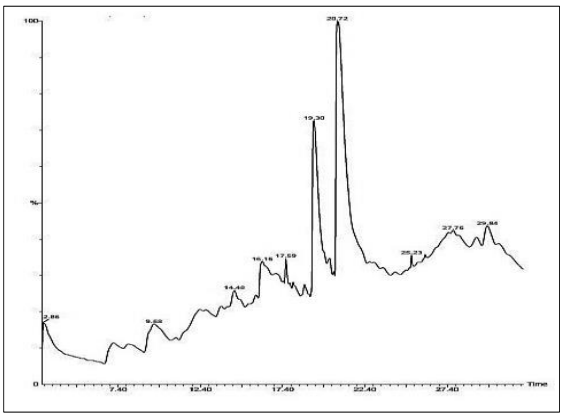

Fig.10 GC-MS analysis for ethanol stem extract of the plant sample

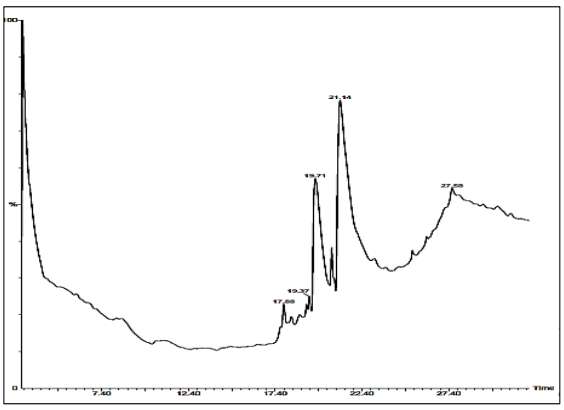

Table.6 GS-MS analysis for methanol leaf extract of the plant sample.

Table.7.GC-MS analysis for ethanol leaf extract of the plant sample

S. No	RT	Compound	Molecular Formula	M.W	% Of Area	Biological Activity
1	17.86	Phytol	C20H40O	296	25.964	Anti-inflammatory Activity
2	18.385	3,7,11,15-Tetramethyl-2-Hexadecen-1-Ol	C20H40O	296	19.015	Anti-inflammatory Activity
3	19.78	Pentacosanoic Acid, 2,10-Dimethyl-, Methyl Ester	C28H56O2	424	1.383	Membrane Integrity/Stability, Cell Signaling
4	20.271	17-Pentatriacontene	C35H70	490	2.029	Unknown
5	25.308	Squalene	C30H50	410	14.773	Unknown
6	27.828	2h-1-Benzopyran-6-Ol, 3,4-Dihydro-2,5,7,8-Tetramethyl-2-(4,8,12- Trimethyl	C31H52O3	472	3.641	Anti-Oxidant activity, Vitamin
7	30.034	2r-Acetoxymethyl-1,3,3-Trimethyl-4t-(3-Methyl-2-Buten-1-Yl)-1t-Cycloh	C17H30O3	282	33.196	Anticancer Activity

8	17.97	16-Heptadecenal	C17H32O	252	5.089	Anticancer activity
9	19.53	1-Octadecyne	C18H34	250	11.749	Unknown
10	21.935	2-Methyl-Z-4- Tetradecene	C15H30	210	26,14	Unknown

Table.8. GC-MS analysis for methanol stem extract of the plant sample.

S.No	RT	Compound	Molecular Formula	M.W	% Of Area	Biological Activity
1	16.149	1-Tetradecanamine	C14H31N	213	5.506	Unknown
2	19.27	N-Hexadecanoic Acid	C12H22O11	342	25.368	Tissue Adhesive, Growth Regulator, Fixative, Tolerogen
3	20.775	9,12-Octadecadienoic Acid (Z,Z)-	C18H32O2	280	69.126	Tissue adhesive, Anticancer activity, Antimicrobial activity
4	18.427	Sucrose	C12H22O11	342	22.34	Anticancer activity, Growth Regulator, Tissue Adhesive, Antimicrobial,
5	17.491	Tetradecanoic Acid	C14H28O2	228	7.32	Tissue Adhesive, Antimicrobial activity, Growth Regulator, Fixative
6	21.281	9-Octadecyne	C18H34	250	2.673	Unknown
7	20.233	Adenosine, N6- Phenylacetic Acid	C18H19O6 N5	401	18.643	Unknown
8	19.67	Tridecanoic Acid	C13H26O2	214	31.568	Tissue Adhesive, Antimicrobial activity.
9	18.113	(Z)6-Pentadecen-1-Ol	C15H30O	226	16.37	Unknown

Table.9 GC-MS analysis for ethanol stem extract of the plant sample

S.No	Rt	Compound	Mol Formula	Mol Wt	% Of Area	Biological Role
1	17.87	Hexadecanal	C16H32O	240	2.027	Unknown
2	19.705	N-Hexadecanoic Acid	C16H32O2	256	32.656	Antibacterial, Antimicrobial, Inhibitory Activity
3	20.646	1-Hexyl-2- Nitrocyclohexane	C12H23O2N	213	2.248	Unknown
4	21.216	1,19-Eicosadiene	C20H38	312	61.169	Tissue Adhesive, Immunomodulatory action, Antimicrobial Activity
5	27.589	Cyclotrisiloxane, Hexamethyl	C6H18O3Si 3	222	1.9	Antibacterial Activity, Antioxidant Activity
6	18.88	Octadecanal	C18H36O	268	3.6	Unknownl
7	19.12	Eicosanoic Acid	C20H40O2	312	23.14	Antimutagen
8	20.334	1-Octadecyne	C18H34		1.78	Unknown
9	23.76	Z,E-2-Methyl-3,13-Octadecadien-1-Ol	C19H36O	280	3.92	Anticancer Activity, Tissue Adhesive
10	24.182	1,2-Bis(Trimethylsilyl)Benzene	C12H22Si2	222	18.4	Unknown

Antioxidant activity for the stem and leaf extracts of *Alternanthera brasiliana kuntz.*

DPPH-Antioxidant assay

The present model of scavenging the stable DPPH radical is widely used method to evaluate the free radical scavenging ability of various samples. DPPH is stable nitrogen centered free radical where the colour changes from violet to yellow upon reduction by either the process of hydrogen or electron donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers.

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Fig.11 DPPH antioxidant activity of *Alternanthera brasiliana*

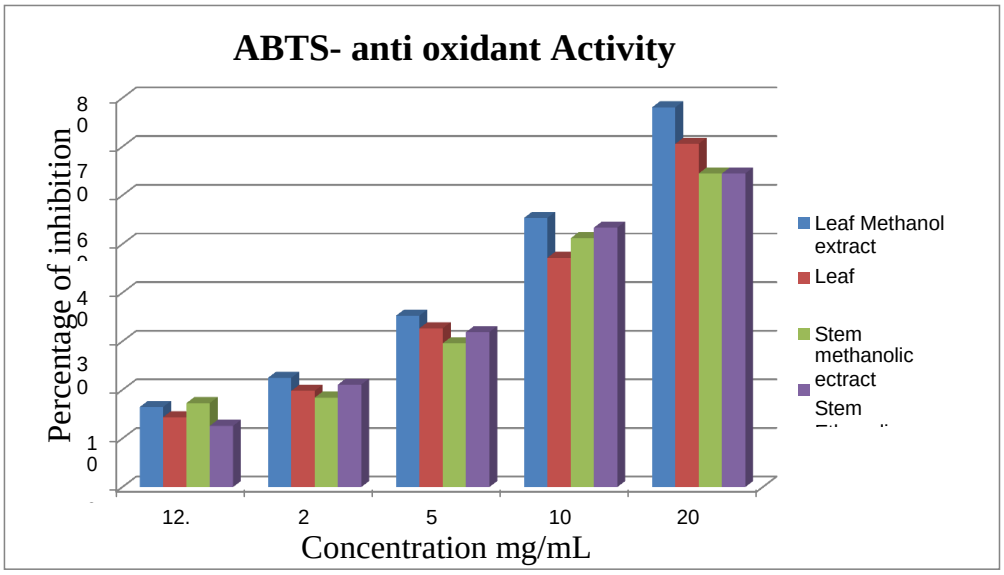

Table.10. DPPH Antioxidant activity of stem and leaf extracts (methanolic and ethanolic) of Alternanthera brasiliana

concentration mg/mL	Leaf		Stem	
	Methanol extract	Ethanol extract	methanolic extract	Ethanolic extract
12.5	16.5	14.4	17.3	12.6
25	22.5	19.8	18.4	21.1
50	35.3	32.7	29.6	31.9
100	55.4	47.2	51.2	53.4
200	78.1	70.6	64.5	64.5

ABTS-Antioxidant assay

The ABTS antioxidant value and it is well documented that flavonoids and polyphenols are natural antioxidants. Flavonoids directly react with superoxide anions and lipid peroxyl radical and consequently inhibit or break the chain of lipid peroxidation. This radical scavenging activity of extracts could be related to the antioxidant nature of polyphones or flavonoids, thus contributing to their electron/ hydrogen donating ability [31].

Fig.12 ABTS antioxidant activity percentation of methanolic and ethanolic extracts of stem and leaf alternanthera brasiliana

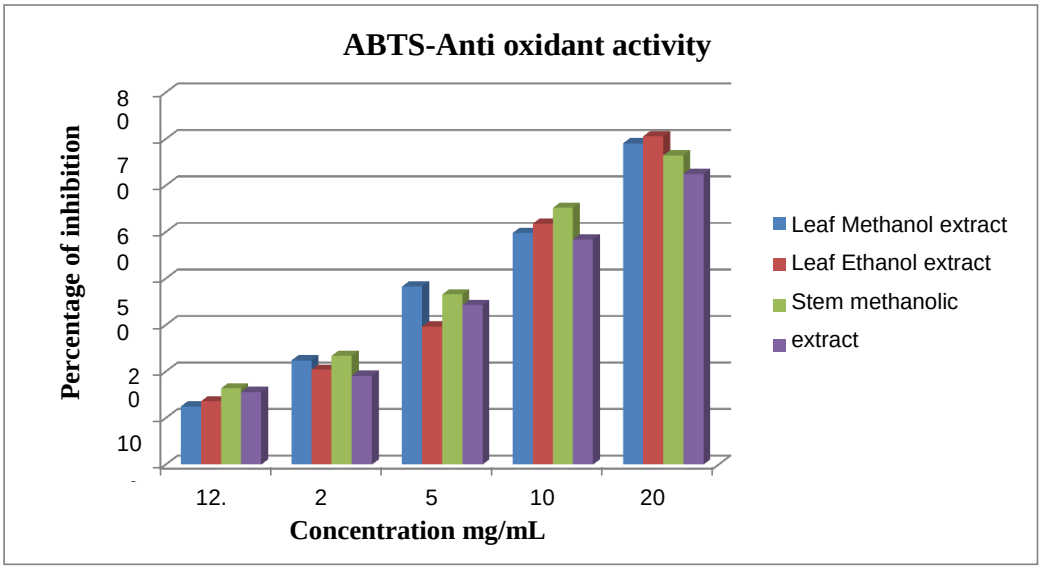

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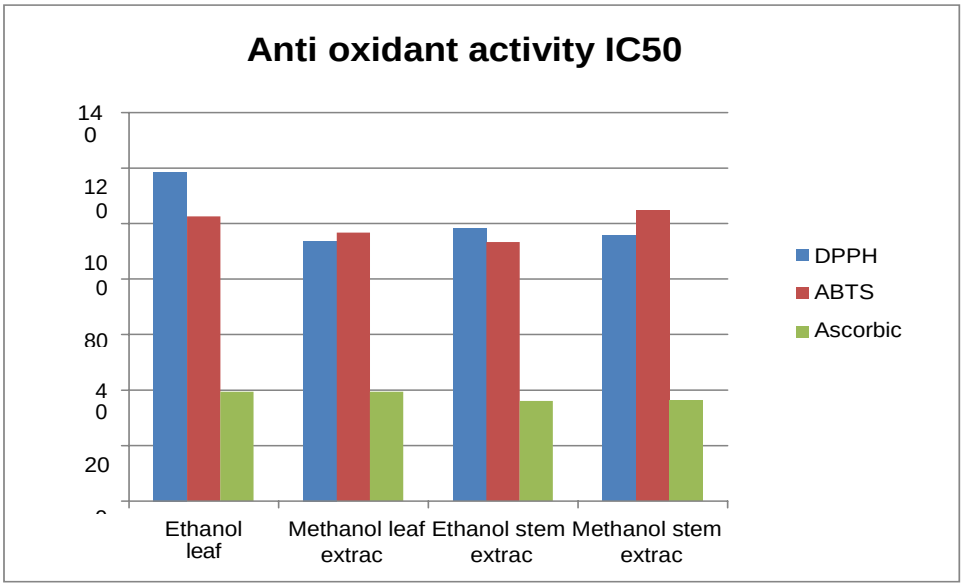
Table.11. ABTS antioxidant activity percentation of methanolic and ethanolic extracts of stem and leaf alternanthera brasiliana

Concentration mg/mL	Leaf		Stem	
	Methanol extract	Ethanol extract	methanolic extract	Ethanolic extract
12.5	12.5	13.6	16.4	15.6
25	22.4	20.4	23.4	19.1
50	38.3	29.7	36.6	34.3
100	49.8	51.8	55.2	48.4
200	69.1	70.6	66.5	62.5

Table.12. IC_{50} concentration of stem and leaf extracts of *Alternanthera brasiliana*

S.No	samples	DPPH	ABTS
1	Ethanol leaf extract	118.5	102.6
2	Methanol leaf extract	93.8	96.7
3	Ethanol stem extract	98.3	93.3
4	Methanol stem extract	95.7	104.7
5	Ascorbic acid	39.46	36.13

Fig.13 IC_{50} values of plant sample in both DPPH and ABTS antioxidantassay



A. brasiliana was evaluated for its potential anti-oxidant activity using using 1,1-diphenyl- 2-picrylhydrazyl (DPPH) radical-scavenging, iron (II)- chelating, nitric oxide radical-scavenging, ferrous sulphate and carbon tetrachlorideinduced lipid peroxidation assays. The percentage inhibition of DPPH radical exhibited by the increasing concentrations of the extract, percentage inhibitions of ferrous sulphate and carbon tetrachloride-induced lipid peroxidation by the extract ranged from 96.29% to 99.59%, 51.43% to 78.78%, 53.43% to 94.85%, 25.00% to 37.90% and 96.26% to 99.50% respectively. The established ethanol extract of the leaves of A. brasiliana is rich in natural anti-oxidants [32]

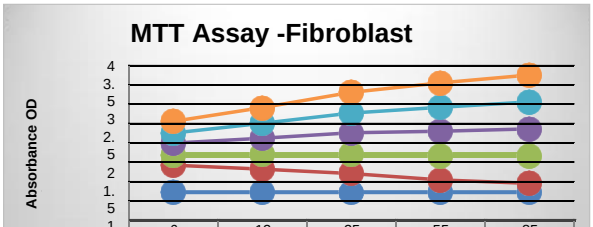
Inorder to compare the present study with earlier study DPPH antioxidant activity of methanol and ethanol extracts of leaf of*Alternathera brasiliana* respectively showed the value of IC_{50} = 93.8 µg/ml and IC_{50} = 118.5 µg/ml and DPPH antioxidant activity of methanol and ethanol extracts of stem showed the value of IC_{50} = 95.7 mg/ml and IC_{50} = 98.3 mg/ml respectively. The antioxidant activity of leaf and stem extracts of *Alternanthera brasiliana* was determined by using ABTS antioxidant assay. The resuls are revealed the extrats showing antioxidant activity when increase in concentration. The ethanolic and methanolic leaf extracts showed the value of IC_{50} = 102.6 mg/ml and IC_{50} = 96.7 mg/ml respectively. Comparatively the methanolic and ethanolic stem extrats also showed the equal percentade of inhibition and the IC_{50} values are 104.7 mg/ml and 93.3 mg/ml respectively [33]

MTT-Based Cell Proliferation Assay [35] [36]

The effect of stem and leaf extracts (ethanolic and methanolic) of *Alternanthera barasiliana* on chick embryo fibroblast cell culture isolated and cultured from 15 days old chick embryo at doses of 6, 12,25,55 and 85 µg/mL. The absorbance was taken at 517 nm wave length which is represented in Fig 14.

e-ISSN: 2582-7405

Fig.14.MTT assay of stem and leaf extracts of *A.brasiliana.*



	6	12	25	55	85
LM	0.305	0.405	0.537	0.63	0.697
LE	0.256	0.386	0.506	0.616	0.697
SM	0.31	0.41	0.542	0.635	0.702
SE	0.26	0.39	0.51	0.62	0.701
Colchicine	0.691	0.597	0.49	0.32	0.223
Control	0.741	0.729	0.726	0.734	0.74

In leaf extracts of *Alternathera brasiliana* methanolic extract showing maximum absorbance OD value at dose of 85 µg/mL and 55 µg/mL the OD values are 0.697 and 0.63 respectively. The ethanolic extract shows maximum OD values at doses of 55 and 85 µg/mL. The least OD value shows at 6 µg/mL concentration. Wich shows increasing proliferation of fibroblast cells according to the increasing dose (µg/mL) of leaf extracts. From the stem extracts of *Alternanthera brasiliana* the methanolic stem extract showing maximum absorbance OD value at doses of 85 and 55 µg/mL and the minimal OD value at dose of 6 µg/mL. The ethanolic stem extract showing maximum absorbance OD value at 85 µg/mL concentration and the OD value is 0.702. At the dose value of extracts 85µg/mL. The both stem extracts showing greater absorbance OD value compared to the both leaf extracts.

Results revealed that the both stem and leaf extracts of *Alternanthera brasiliana* significantly enhanced the proliferation rate of chick fibroblast at concentration less than 85µg/mL. The selection of the plant agent was based on its traditional medicine use and wound healing activity in animal model and in different types of wounds reported.

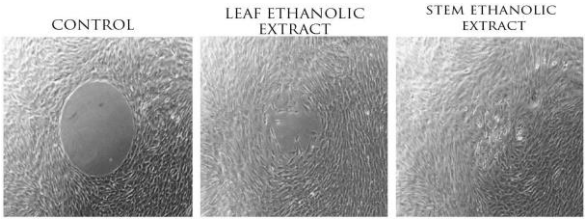
Wound Healing Activity

The chick embryo fibroblast cells were isolated and cultured in 24-well plate. The layer of fibroblast cells grown after 48 hours incubation. The cells were rubbed and wound was made in cell lines. The addition of colichine in fibroblast cells which result senescence. The wounded cells are incubated for 48 hours incubation and observe the cells migrating and wound closure in microscope. Wound healing activity is determined by using ethanolic and methanolic extracts of stem and leaf of *Alrenathera brasiliana* separately at different Concentrations of 6, 12, 25, 55, 85µg/mL. The microscopic views at particular time intervals show the maximum wound healing property of stem and leaf extracts in different concentration (Fig 15).

Table.13. ED_{50} concentration of leaf and stem extracts of *Alternanthera Brasiliana*

S. No	Plant Parts	Extract	Ed_{50} Concentration(µg/Ml)
1	Leaf	Ethanol	55
		Methanol	85
2	Stem	Ethanol	55
		Methanol	85

Fig.15 Wound healing activity of ethanolic extract of stem and leaf of *Alternathera brasiliana*



The study shows both the stem and leaf extracts of *A.brasilian* has wound healing potential. Methanolic and ethanolic extracts of leaf of *A.brasiliana has* wound healing activity at each concentration and the 50% effective dosage (ED_{50}) at the concentration 85 and 55 µg/mL respectively. The ethanolic extracts of leaf shows maximum wound healing property compared to the mathanolic extract of leaf. Methanolic and ethanolic extracts of stem of *A.brasiliana* has wound healing activity at concentration ≥ 25 and the 50% effective dosage (ED_{50}) at the concentration 85 and 55 µg/mL respectively. The ethanolic extracts of stem shows maximum wound healing property compared to the other extract.

DISCUSSION

The present study revealed that the both stem and leaf showing wound healing activity. From the extracts the ethanolic extracts showing great wound healing potential at a time interval. Ethanolic stem extracts shows comparatively higher wound healing potency (Table.13 and Fig.15). Phytochemical screening results and the biologically active compound identification help to rectify the results and which is evidence for wound healing property of extracts. The stem and leaf extract of *Alternathera brasiliana* are obtained through cold extraction method and from the both plant parts maximum extract obtain from methanol solvent in both stem and leaf and the amount of extract obtain was 2110 (mg/60gm) and 1840 (mg/60gm) respectively, Comparatively the extract obtained from leaves are in higher amount. The qualitative analysis of phytochemicals in extracts results in presence different metabolites including alkaloids, phenols, flavonoid, saponins and glycoside. The phytochemical screening of extracts obtained from stem and leaf of *Alternathera brasiliana* reveals the presence of characteristic functional group including aldehydes, carboxylic acids, esters, amines, alkenes, alkanes and carboxylic acids. The GC-MS analysis of the extracts showing presents more than 40 compounds. In leaf ethanolic extract 16- heptadecenal and 3,7,11,15,

tetramethyl-2-hexaden-1-ol are in high amount. The compound 2r-Acetoxymethyl-1,3,3-Trimethyl-4t-(3-Methyl-2-Buten-1-Yl)-1t-Cycloh was identified in ethanol extract of leaf with area % 33.196. GC-MS analysis of methanol stem extract 9,12 octadedienoic acid (Z,Z) and tridecanoic acid are more area % and the compounds1,9 eicosadiene (61.169 are %) and N-hexadecanoic acid (32.656 area %) are identified in ethanolic extract of stem. These cmpounds shows biological activities like anticancer activity and tisuue adhesive activity. The tissue adhesive property of compound help and influencing wound healing result in reduce in healing time. Dermabond is a cyanoacrylate tissue adhesive that forms a strong bond across apposed wound edges, allowing normal healing to occur below. It is marketed to replace sutures that are 5-0 or smaller in diameter for incisional or laceration repair. This adhesive has been shown to save time during wound repair, to provide a flexible water-resistant protective coating and to eliminate the need for suture removal [23].

A. brasiliana was evaluated for its potential anti-oxidant activity using using 1,1-diphenyl- 2-picrylhydrazyl (DPPH) radical-scavenging, iron (II)- chelating, nitric oxide radical-scavenging, ferrous sulphate and carbon tetrachlorideinduced lipid peroxidation assays. The percentage inhibition of DPPH radical exhibited by the increasing concentrations of the extract, percentage inhibitions of ferrous sulphate and carbon tetrachloride-induced lipid peroxidation by the extract ranged from 96.29% to 99.59%, 51.43% to 78.78%, 53.43% to 94.85%, 25.00% to 37.90% and 96.26% to 99.50% respectively. The established ethanol extract of the leaves of A. brasiliana is rich in natural anti-oxidants (Osmund *et al.*, 2013). Inorder to compare the present study with earlier study DPPH antioxidant activity of methanol and ethanol extracts of leaf and stem of*Alternathera brasiliana* shows more great antioxidant activity with increase in concentration. In the study of leaf methanol and ethanol determined values are IC_{50} = 93.8 mg/ml and IC_{50} = 118.5 mg/ml and DPPH antioxidant activity of methanol and ethanol extracts of stem showed the value of IC_{50} = 95.7 mg/ml and IC_{50} = 98.3 mg/ml respectively. The determination of antioxidant activity of leaf and stem extracts of *Alternanthera brasiliana* by using ABTS antioxidant assay, revealed the extracts was increased in concentration. The ethanolic and methanolic leaf extracts showed the value of IC_{50} = 102.6 mg/ml and IC_{50} = 96.7 mg/ml respectively. Comparatively the methanolic and ethanolic stem extracts also showed equal percentage of inhibition and the IC_{50} values are 104.7 mg/ml and 93.3 mg/ml respectively. In the MTT based cell proliferation assay, the ethanolic extracts of leaf and stem shows maximum proliferation at a concentration 55 µg/ml. which was concluded with OD value shown in particular concentration (Fig.14).

Wound healing activity of methanolic extract of leaves of *Alternanthera brasiliana* Kuntz was studied by excision and incision wound model (in vivo) in Sprague Dawley rats and by Chorioallantoic membrane (CAM) model (In vitro) in 9-day-old embryonated chicken eggs. The result suggested that methanolic extract of *A.brasiliana* possess significant wound healing potential in normal wound. Methanolic extract of *Alternanthera brasiliana* has significant increase angiogenesis and tensile strength [14]. Wound healing activity of methanol extract of *Alternanthera brasiliana* [5% (w/w) ointment] was evaluated in experimental burn wound model in rats. Healing potential was assessed by the rate of wound contraction along with histopathological examination. There was a significant increase in wound contraction along with augmented level of antioxidants in granulation tissues in *A. brasiliana* treated group. Histopathological assessment of the granulation tissue shows formation of epidermis with keratin layer and deposition of collagen fibers and triterpenes are known to promote the wound healing process mainly due to their astringent and antimicrobial property [6]. The constituents like triterpenes and alkaloids of *A. brasiliana* may also play major role in the process of wound healing and *A. brasiliana* exhibited significant wound healing activity in immune-compromised wound as evidenced by augmented endogenous antioxidants and increased angiogenesis.

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