ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: https://ijrps.com

Antioxidant and antimicrobial activity of methanolic extract of aerial parts of *Alternanthera bettizickiana*

Ramakrishnan Padmini*[1], Chandragiri Janakiraman Thirupurasundari[2], Subramania Bharathiraja[2], Sivasithambaram Niranjali Devaraj[2]

[1]Department of Biochemistry, School of life sciences, VELS Institute of Science Technology and Advanced Studies (VISTAS), Chennai, India
[2]Department of Biochemistry, University of Madras, Chennai, India

Article History:

Received on: 15.02.2018
Revised on: 23.05.2018
Accepted on: 26.05.2018

Keywords:

Antioxidant, Antimicrobial, Hepatotoxicity, *Alternanthera bettizickiana*

ABSTRACT

The study aimed to carry out the *in vitro* antioxidant and antimicrobial activities of the methanolic extract of aerial parts of *Alternanthera bettzickiana* (ABME). ABME showed its ability to scavenge the free radicals in a concentration-dependent manner. The plant extract (100 mg) yielded 92.6±0.13mg/ml gallic acid-equivalent phenolic content and 280.5±0.12 mg/ml quercetin-equivalent flavonoid content. ABME showed total antioxidant activity with a Trolox equivalent antioxidant concentration (TEAC) value of 0.77±0.03. The IC_{50} values for scavenging of free radicals were 292.578µg/ml, 212.582 µg/ml, 146.1 µg/ml and 434.9µg/ml for DPPH, hydroxyl, superoxide and nitric oxide respectively. The antimicrobial activity was tested against strains of *Escherichia coli, Pseudomonas aeruginosa*, *Staphylococcus aureus, Staphylococcus epidermidis*, *Candida albicans* and *Aspergillus niger*. ABME was found to be effective against these tested organisms. The present study provides evidence that aerial parts of *Alternanthera bettzickiana* are a potential source of natural antioxidants with antimicrobial properties.

* Corresponding Author

Name: Ramakrishnan Padmini
Phone: +91-9941165319
Email: minikrishnan30@gmail.com

ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v9i3.1613

Production and Hosted by

IJRPS | https://ijrps.com

INTRODUCTION

Herbal medicine is the mainstay of about 75-80% of the whole population and the major part of traditional therapy involves the use of plant extract and their active constituents (Akerele, 1993). Human infections constitute a severe problem and most frequent pathogens are microorganisms such as bacteria and fungi. The fungal infections in the immunocompromised individuals have significantly increased in the recent years (Wheat 1994) such as neutropenia, leukopenia, chronic admin- and these infections include important risk factors

istration of corticosteroids and other antifungal agents, hepatotoxicity, cutaneous reactions and tissue lesions (Pujol, 1996). Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic an- timicrobials (Agarwal *et al*., 1996).

Alternanthera bettizickiana belongs to the Ama-ranthaceae family. The family comprises many species with biological activities, which are used in nutrition and alternative medicine (Gorinstein *et al*., 1991, Salvador *et al*., 2002, Siqueira, 1987). This family includes approximately 65 genera and 1000 species. Many species of *Alternanthera* are used in the treatment of infections, as an analgesic, anti-nociceptive, anti-viral and diuretic (Gor-instein *et al*., 1991).

The plant is commonly called as "Red calico plant". The leaves are said to be edible raw or cooked. This is the first report on the antioxidant and antimicro- bial properties of *Alternanthera bettizickiana.*

Some species of genus *Alternanthera* are thought to possess antimicrobial and antiviral properties (Salvador *et al*., 2002, Jalalpure, Agrawal, Patil, Chimkode & Tripathi, 2008). In Brazilian folk medicine, the aqueous extract of *A. tenella* colla is used for its anti-inflammatory activity (Guerra, Pereira, Silveira and Olea, 2003). There is no report of biological studies in the literature about *Alternanthera bettzickiana*, however, in a preliminary evaluation, the crude extracts of adults plants have shown good antimicrobial activity. Therefore, the present work reports the results of the comparative study of antioxidant and antimicrobial activity.

MATERIAL AND METHODS

Chemicals and their sources

Di Picryl Phenyl Hydrazine (DPPH) from Sigma Aldrich, USA. 2,2'-azinobis-(3-ethylbenzothiazoline- 6-sulfonic acid) (ABTS) was obtained from Roche Diagnostics, Mannheim, Germany. 6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid (Trolox) was obtained from Fluka, Buchs, Switzerland. Peptone, potato dextrose agar, Beef extract, Yeast extract, agar – were purchased from Himedia. NaCl, Ascorbic Acid, Thiobarbituric acid, Butylated Hydroxy Anisole (BHA) from SRL, Mumbai. All other chemicals and solvents used were of analytical grade.

Microorganisms

The investigated microorganisms consisted of Gram-positive bacteria: *Staphylococcus aureus* ATCC25923, *Staphylococcus epidermidis* ATCC12228; Gram-negative bacteria: *Pseudomonas aeruginosa* ATCC27853, *Escherichia coli* ATCC25922 and fungi: *Candida albicans* ATCC2091, *Aspergillus niger* ATCC 1015. Microorganisms were obtained from the National Chemical Laboratory (NCL), Pune, India.

Plant Material

The plant material *Alternanthera bettizickiana* was collected in and around Chennai and authenticated by Dr. P. Jayaraman, Plant Anatomy Research Centre, Chennai, India.

Preparation of Plant Extracts

The aerial parts of *Alternanthera bettizickiana* were gabled for removal of adulterants and pulverized. It was air dried at room temperature and one hundred grams (100g) of the pulverized leaves was exhaustively defatted with petroleum ether (60-80º) and successively extracted with chloroform, ethyl acetate and then finally methanol using the Soxhlet extractor. The extract portion of methanol was concentrated *in vacuo* and a brown mass that weighed twenty grams (20.52g) equivalent to 20.50 % (w/w) obtained. The methanol extract was kept aseptically until use.

Total Phenolic Constituents Study

The total phenolic content was determined by the method of McDonald *et al*., 2001 and then expressed as gallic acid equivalents.

Total Flavonoids Determination

The aluminum chloride colorimetric method was used for flavonoids determination (Chang *et al*., 2002). Varying concentration of plant extract (0.5mL of 1:5 g/mL) was mixed separately with 1.5 ml of methanol, 0.1ml of 10% Aluminium chloride, ml of 1M potassium acetate and 2.8ml of distilled water. It remained at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415nm. The calibration curve was prepared by preparing quercitin solutions at concentrations 12.5 to 100 µg/ml in methanol.

Flavonoids Determination by HPLC Analysis

The flavonoids were extracted using the extraction solvent - a mixture of alcohol, water and HCl (50:20:8) and mobile phase includes a mixture of methanol, water and phosphoric acid (100:100:1). The liquid chromatograph is equipped with a 270nm detector and a 4.6-mm x 25cm column that contains packing L1. The flow rate is about 1.5ml per minute. Standard solutions used were quercetin, kaempferol and isorhamnetin.

Evaluation of Antioxidant Activity

Total Antioxidant Activity

The ability of the test sample to scavenge $ABTS^{\cdot+}$ radical cation was compared to Trolox standard (Re *et al*., 1999). ABTS was dissolved in deionized water to 7 mM concentration and potassium persulphate added to a concentration of 2.45 mM. The reaction mixture was left to stand at room temperature overnight (12 to 16 h) in the dark before usage. The resultant intensely-coloured ABTS•+ radical cation was diluted with 0.01 M PBS (phosphate buffered saline), pH 7.4, to give an absorbance value of ~0.70 at 734 nm. Then 1 ml was mixed with 10 µl of the test sample (10–100µg/ml) and the absorbance was measured at 734 nm after 6 min. The Trolox equivalent antioxidant capacity was subsequently calculated.

DPPH Radical Scavenging

The free radical scavenging capacity of the methanolic extracts of *Alternanthera bettizickiana* was determined using DPPH by the method of Sandoval M *et al.,* 2000. To 1 ml of various concentrations of extract, 1 ml solution of DPPH 0.1mM was added. An equal amount of methanol

and DPPH served as control. Ascorbic acid was used as positive control. After 20 min incubation in the dark, absorbance was recorded at 517 nm. The degree of DPPH radical scavenging activity of antioxidants was calculated as % of inhibition by the following expression:

% Inhibition = $[(A_{control} - A_{sample})/A_{control}] * 100$

Where $A_{control}$ is an absorbance at time = 0, and A_{sample} is an absorbance at time = 5 min.

NBT (Superoxide Scavenging) Assay

The superoxide anion radical scavenging activity was assayed as described by Liu and Ng (2000). In brief, varying concentration of methanolic extract was treated with 3.0 ml of Tris-HCl buffer (16 mM, pH 8.0), which contained 78 µM (β-nicotinamide adenine dinucleotide (reduced form, NADH), 50µM nitroblue tetrazolium (NBT), 10µM phenazine methosulfate (PMS). The colour reaction of superoxide radicals and NBT was detected at OD 560nm and ascorbic acid was used as positive control. The inhibition ratio (%) was calculated as % of inhibition = [(absorbance of control - absorbance of test sample)/absorbance of control] x 100%.

Hydroxy Radical Scavenging Assay

The reaction mixture, containing extract (10–100µg/mL), was incubated with deoxyribose (3.75 mM), H_2O_2 (1 mM), $FeCl_3$ (100 µM), EDTA (100 µM), and ascorbic acid (100 µM) in potassium phosphate buffer (20 mM, pH 7.4) for 60 min at 37°C. The reaction was terminated by adding 1 mL TBA (1% w/v) and 1 mL TCA (2% w/v) and then heating the tubes in a boiling water bath for 15 min. The contents were cooled and the absorbance of the mixture was measured at 535 nm against reagent blank. The decreased absorbance of the reaction mixture indicated decreased oxidation of deoxyribose (Halliwell, Gutteridge and Auroma, 1987).

Nitric Oxide Scavenging Assay

Sodium nitroprusside, 5mM, was prepared in phosphate buffer pH 7.4. To 1 ml of various concentrations of test compound, sodium nitroprusside 0.3 ml was added. The test tubes were incubated at 25°C for 5 h. After 5 h, 0.5 ml of Griess reagent was added. The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with *N*-(1) naphthyl ethylenediamine was read at 546 nm. (Sreejayan and Rao, 1997).

Antimicrobial Activity

Microorganisms were maintained at 4°C on nutrient agar slants. For the bacterial strains, overnight cultures grown in nutrient broth were adjusted to an inoculum size of 10^8 cells/ml for inoculation of the agar plates. An aliquot (0.2 ml) of inoculum was added to the nutrient agar medium (HiMedia). A final inoculum containing 10^4 spores/ml of fungi spread on Potato Dextrose Agar (PDA) medium was used for the fungal strains.

The agar well diffusion assay (Mohanasundaram *et al*., 2017) was used to determine the growth inhibition of bacteria by the plant extract. Bacteria were maintained at 37°C on nutrient agar plates before use. Nutrient agar was prepared and 25ml each was poured into a sterile petri dish. This was allowed to solidify and dry. Using a sterile cockborer of 8mm diameter three equidistant holes per plate were made in the agar and were inoculated with a 0.2ml overnight suspension of the bacteria. The wells (holes) were filled with the extract solution at varying concentrations of 10 mg/ml, 5 mg/ml and 2.5 mg/ml respectively. The plates were incubated at 37ºC for 24 hours. The experiments were repeated thrice. The antibacterial activities were observed and measured using a transparent meter rule and recorded if the zone of inhibition was ≥10mm (Vlietink *et al*., 1995 and Kudi *et al*., 1999). Similarly, the antifungal activities were evaluated.

Determination of MIC_{50}

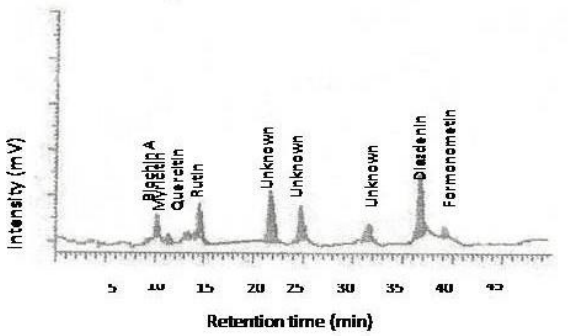
The minimum inhibitory concentrations of plant extracts were determined by serial dilution in the nutrient agar, with concentrations ranging from 5, 10, 20, 25, 50, 75, 100, 125, 150, 175, 200, 225 and 250µg/mL. The inoculum was prepared from fresh overnight broth culture in nutrient broth. Plates were incubated for 24 hr at 37°C. The MIC was recorded as lowest extract concentration demonstrating no visible growth in the broth. (Baykam *et al*., 2004)

Statistical Evaluation

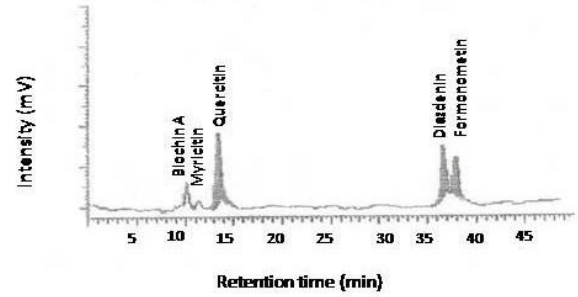
All results are expressed as mean ± S.E.M (n = 6). IC_{50} values were calculated by applying suitable regression analysis from the mean inhibitory values.

RESULTS AND DISCUSSION

Many crude extracts and active principles derived from plant species of *Alternanthera* have been studied for antibacterial, wound healing, antihypertensive activity (Hosamani, Ganjihal, Chavadi, 2004). The results of our screening assays also confirmed the use of *Alternanthera bettizickiana* in traditional medicine. It is the first report about antimicrobial and antioxidant effects of aerial parts of *Alternanthera bettizickiana.*



a) HPLC fingerprint of flavonoids of ABME



b) HPLC fingerprint of Standard flavonoids

Figure 1: Determination of flavonoids content by HPLC analysis

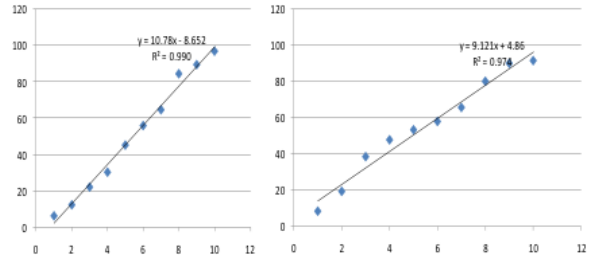


Figure 2: ABTS free radical scavenging activity

Antioxidant activity of different concentrations of a) ABME and b) reference compound Trolox on decolorization of ABTS radical cation. The percentage of inhibition was plotted against the concentration of the sample. Each value represents mean ± S.E.M (n=6).

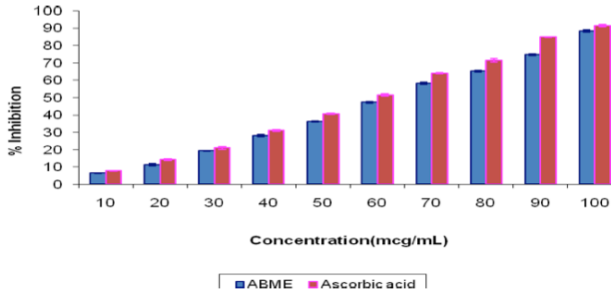


Figure 3: DPPH free radical scavenging activity

Antioxidant activity of different concentrations of ABME and ascorbic acid in DPPH radical scavenging. The results are expressed in terms of concentration *vs. %* scavenging. Each value represents mean ± S.E.M

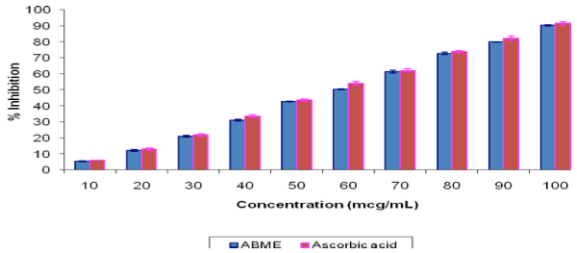


Figure 4: Superoxide scavenging assay

Superoxide anion radical scavenging activity of different concentrations of ABME and ascorbic acid by NBT method. The results are expressed in terms of concentration *vs. % scavenging. Each value represents mean ± S.E.M.*

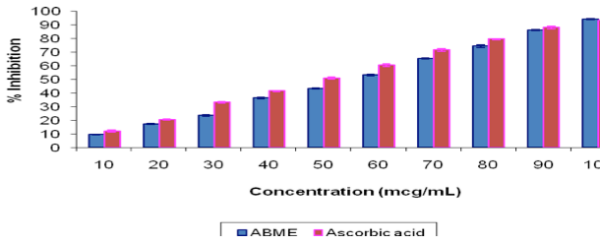


Figure 5: Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity of different concentrations of ABME and ascorbic acid by NBT method. The results are expressed in terms of concentration vs. % scavenging. Each value represents mean ± S.E.M.

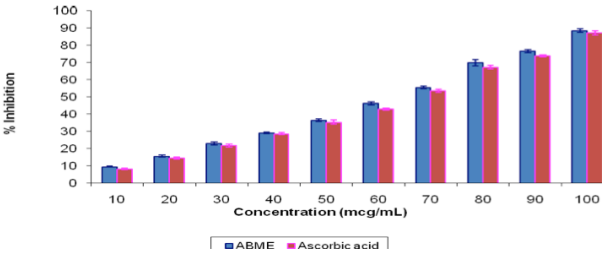


Figure 6: Nitric oxide scavenging assay

Antioxidant activity of different concentrations of ABME and ascorbic acid in nitric oxide radical scavenging. The results are expressed in terms of concentration *vs. %* scavenging. Each value represents mean ± S.E.M.

TOTAL PHENOLICS, FLAVONOIDS CONTENT AND HPLC ANALYSIS

Phenolic compounds may contribute directly to antioxidative action. The total phenolic content was 92.6 ± 0.13 mg/ml of gallic acid equivalent per 100 mg plant extract. The total flavonoid content of the methanolic extract of *Alternanthera bettizickiana* was 280.5 ± 0.12 mg/ml quercetin equivalent per 100 mg plant extract. The major flavonoids detected by HPLC include Biochin A 0.335%, Myricetin 0.1145%, Quercitin 0.125%, Rutin 0.0675% Diadzein 0.0896% Formononetin 0.056% (figure 1).

ABTS Free Radical Scavenging Activity

ABTS•+ is a blue chromophore produced by the reaction between ABTS and potassium persulfate. The decolorization of ABTS•+ cation radical is an unambiguous way to measure the antioxidant activity of phenolic compounds. Addition of the plant extract to this pre-formed radical cation reduced it to ABTS in a concentration-dependent manner. Thus, the ability of a compound to scavenge ABTS•+ radical can demonstrate oxygen radical absorbance capacity. The results were compared with those obtained using Trolox and the TEAC value demonstrates that the extract is a potent antioxidant (figure 2). Awika *et al*., (2004) found positive correlations between phenolic content and antioxidant activity tested using the Oxygen Radical Absorbance Capacity (ORAC), ABTS and the 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) assays. The mechanism of action of flavonoids is through scavenging or chelation. Phenolic compounds are also very important plant constituents because their hydroxyl groups confer scavenging ability (Yildirim *et al*., 2000). Flavonoids are chemically one-electron donors. They serve as derivatives of conjugated ring structures and hydroxyl groups that have the potential to function as antioxidants in *vitro* cell culture or cell-free systems by scavenging superoxide anion, singlet oxygen, lipid peroxy-radicals, and/or stabilizing free radicals involved in oxidative processes through hydrogenation or complexing with oxidizing species. The capability to interact with protein phosphorylation and the antioxidant, iron-chelating and free radical scavenging activity may account for the wide pharmacological profile of flavonoids (Saija *et al*., 1995).

DPPH Free Radical Scavenging Activity

The free radical scavenging activity of methanolic extract of *Alternanthera bettizickiana* was studied by its ability to bleach the stable radical DPPH (figure 3). The radical scavenging activity was compared with the known antioxidant ascorbic acid. This assay provides information on the reactivity of compounds with a stable free radical. Because of the odd electron, DPPH shows a strong absorption band at 517 nm in visible spectroscopy. As this electron becomes paired off in the presence of free radical scavenger, the absorption vanishes, and the resulting decolorization is stoichiometric with respect to the number of electrons taken up (Badmis *et al*., 2003).

Maximum scavenging activity was found at a concentration of 100µg/ml whilst the minimum scavenging activity was found at 10µg/ml (figure 3). The test compound was observed to scavenge free radicals in a concentration-dependent manner between the concentrations of 10-100µg/ml. IC 50 value was 292.578 µg/ml.

From the DPPH assay results, it may be postulated that methanolic extract of *Alternanthera bettizickiana* reduces the radical to the corresponding hydrazine on reacting with the hydrogen donors in the extract. The bleaching of DPPH represents the capacity of *Alternanthera bettizickiana* to scavenge free radicals. The present investigation shows that *Alternanthera bettizickiana* are as effective as ascorbic acid in scavenging DPPH radicals.

Superoxide Scavenging Assay

Alkaline DMSO, used as a superoxide-generating system reacts with NBT to give coloured diformazan. In the presence of scavenger, the reduction of NBT can be measured at 560 nm. Maximum scavenging activity was observed at 100µg/ml with inhibition of 90.46% (figure 4). The IC50 was found to be 146.1µg/ml for ABME and 140.9µg/ml for ascorbic acid.

Hydroxy Radical Scavenging Assays

This assay shows the abilities of the extract and standard mannitol to inhibit hydroxyl radical-mediated deoxyribose degradation in a Fe^{3+}-EDTA-ascorbic acid and H_2O_2 reaction mixture. The results are shown in figure 5. The IC_{50} value of the extract and standard in this assay were 212.5µg/ml and 359 µg/ml, respectively. The IC_{50} value of the extract was less than that of the standard. The activities of antioxidants have been attributed to various mechanisms such as prevention of chain initiation, decomposition of peroxides, reducing capacity and radical scavenging. The observed scavenging activity may be due to the flavonoids present in them.

Nitric Oxide Scavenging Assay

Figure 6 illustrates a significant decrease in the NO radical due to the scavenging ability of extracts and ascorbic acid. We used SNP, which is the spontaneous liberator of NO in the presence of light irradiation. The extracts of *Alternanthera bettizickiana* decreased the amount of nitrite generated from the decomposition of SNP *in vitro.* The scavenging of NO by the extracts was increased in a dose-dependent manner. The extract showed the maximum activity of 87.13% at 100 µg/ml, whereas ascorbic acid at the same concentration exhibited 91.33% inhibition. The IC 50 values were found to be 434.9µg/mL and 109.4µg/ml for ascorbic acid and *Alternanthera bettizickiana* respectively. Sodium nitroprusside serves as a chief source of free radicals. The absorbance of the chromophore

Table 1: Antimicrobial potential of extracts of fruits of Alternanthera bettizickiana in agar well diffusion method and MIC 50 values

Organism	Positive Control	1000µg	500µg	250µg	125µg	Negative control (Methanol)	MIC 50 (µg/ml)
Gram +ve	Tetracycline (30 µg)						
S. aureus	20	17	15	13	11	10	>10
S. epidermidis	19	20	17	12	11	10	>25
Gram –ve							
P. aeruginosa	20	20	18	15	12	11	>100
E. coli	21	19	16	14	11	10	>200
Fungi	Amphotericin B (20 µg)						
C. albicans	23	24	21	18	14	9	>250
A. niger	22	20	19	16	13	10	>200

formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with naphthylethylene diamine is used as a marker for nitric oxide scavenging activity (Balakrishnan, Panda, Raj, Shrivastava and Prathani, 2009). The chromophore formation was not complete in the presence of extracts, which scavenges the NO thus formed from the sodium nitroprusside and hence the absorbance decreases as the concentration of the extract increases in a dose-dependent manner. These values revealed that the antioxidant activity of methanol extract is due to the presence of high content of crude flavonoids and saponins (Ren, Qiao, Wang, Zhu and Zhang, 2003).

ANTIMICROBIAL ACTIVITY AND MIC 50

Table 1 reports the antibacterial and antifungal susceptibility of various concentration of plant extract against Gram-positive bacteria: *Staphylococcus aureus, Staphylococcus epidermidis;* Gram-negative bacteria: *Pseudomonas aeruginosa, Escherichia coli* and fungi: *Candida albicans, Aspergillus niger.* The antibacterial activity is compared with the standard antibiotics. The results show significant activity against investigated species.

ABME showed potential bacterial inhibition with MIC values varying from 10 to 250 µg/ml for *Staphylococcus aureus, Staphylococcus epidermidis;* Gram-negative bacteria: *Pseudomonas aeruginosa, Escherichia coli* and fungi: *Candida albicans, Aspergillus niger.* The plant-based compounds exert their mechanism in either of these ways, Degradation of the cell wall, Damage to the cytoplasmic membrane, Damage to membrane proteins, Leakage of cell contents or Coagulation of cytoplasm and depletion of the proton motive force (Oosterhaven *et al*., 1995). One important characteristic of plant extract and their constituents is their hydrophobicity, which enables them to partition in the lipids of the bacterial cell membrane, disturbing the structures and rendering them more permeable (Knobloch *et al*., 1986). Leakage of ions and other cell contents can then occur (Oosterhaven *et al*., 1995). Although a certain amount of leakage from bacterial cells may be tolerated without loss of viability, extensive loss of cell contents or the exit of critical molecules and ions will lead to death. This mode of antibacterial action is mainly mediated by a phenolic group of flavonoids (Knobloch *et al*., 1986). Thus, the observed antimicrobial action is due to flavonoids and other important secondary metabolites in methanolic extract of *Alternanthera bettizickiana*. Plants from the genus *Alternanthera* are thought to possess antimicrobial and antiviral properties. (Guerra, Pereira, Silveira and Olea, 2003). The demonstration of activity against both gram-negative and gram-positive bacteria is an indication that the plant can be a source of bioactive substances that could be a broad spectrum of activity.

CONCLUSION

Alternanthera bettizickiana demonstrated very strong antioxidant activity, as compared to typical antioxidants (ascorbic acid, BHA and quercetin). The antioxidant and antimicrobial activity is due to their flavonoids, phenolic compounds and their relative chemical structures. The present study supports the potential use of *Alternanthera bettizickiana* as a source of antioxidant and antibacterial compounds.

REFERENCES

Agarwal, P, Rai, V, & Singh, RB, 1996. Randomized, placebo-controlled, single-blind trial of holy basil leaves in patients with non-insulin-dependent diabetes mellitus. *International Journal of Clinical Pharmacology and Therapeutics*, 34, 406-409.

Akerele, O, 1993. Summary of WHO Guidelines for the Assessment of Herbal Medicines. *Herbal Gram* 22,13-28.

Awika, JM, Rooney, LW, Wu, X, Prior RL, & Zevallos, LC 2003**.** Screening methods to measure antioxidant activity of Sorghum (*Sorghum bicolor*) and Sorghum products. *Journal of Agricultural Food Chemistry*, 51 (23), 6657–6662.

Badmis, S, Gupta, MK, & Suresh, B 2003. Antioxidant activity of the ethanolic extract of *Striga orobanchioides. Journal of Ethno Pharmacology*, 85 (24), 227- 230.

Balakrishnan, N, Panda, AB, Raj, NR, Shrivastava, A, & Prathani, R, 2009. The Evaluation of Nitric Oxide Scavenging Activity of *Acalypha Indica* Linn. Root. *Asian Journal of Research Chemistry,* 2(2), 148-150.

Baykam, N, Esener, H, Ergonuln, O, Eren, S, Celikbas AK, & Dokuzouz, B, 2004. *In-vitro* antimicrobial susceptibility of Brucella species. *International journal of antimicrobial agents*, 23 (4) 405-407.

Chang, C, Yang, M, Wen, H, & Chem, J, 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of food and drug analysis,* 10*,* 178-182.

Gorinstein, S, Nue, J, & Arruda, P, 1991. Alcohol-soluble and total proteins from Amaranth seeds and their comparison with other cereals. *Journal of Agricultural Food Chemistry,* 39, 848-850.

Guerra, RNM, Pereira, HAW, Silveira, LMS, & Olea, RSG, 2003. *Brazilian Journal of Medical and Biological Research,* 36(9), 1215-1219.

Halliwell, B, Gutteridge, JMC & Aruoma, OI, 1987. The deoxyribose method: A simple test tube assay for determination of rate constants for reactions of hydroxyl radicals. *Analytical Biochemistry,* 165, 215 -219.

Hosamani, KM, Ganjihal, SS, Chavadi, DV, 2004. Alternanthera triandra seed oil: a moderate source of ricinoleic acid and its possible industrial utilization. *Industrial crops and products*, 19, 133-136.

Jalalpure, SS, Agrawal, N, Patil, MB, Chimkode, R, Tripathi, A, 2008. Antimicrobial and wound healing activities of leaves of *Alternanthera sessilis* Linn. *International journal of green pharmacy,* 2 (3),141-144.

Knobloch, K., H. Weigand, N. Weis, H.-M. Schwarm, and H. Vigenschow. 1986. The action of terpenoids on energy metabolism, p. 429-445. *In* E. J. Brunke (ed.), Progress in Essential Oil Research: 16th International Symposium on Essential Oils. De Gruyter, Berlin.

Liu, F, & Ng, TB, 2000. Antioxidative and free radical scavenging activities of selected medicinal herbs. *Life Sciences* 66, 725-735.

McDonald, S, Prenzler, PD, Autolovich, M, & Robards, K, 2001. Phenolic content and antioxidant activity of olive extracts. Food Chemistry, 73, 73-84.

Mohanasundaram.S, VA Doss, Haripriya G, Varsha M, Daniya S, Madhankumar. GC-MS analysis of bioactive compounds and comparative antibacterial potentials of aqueous, ethanolic and hydroethanolic extracts of Senna alata L against enteric pathogens. Int. J. Res. Pharm. Sci., 2017; 8 (1): 22 – 27.

Oosterhaven, K., B. Poolman, and E. J. Smid. 1995. S-carvone as a natural potato sprout inhibiting, fungistatic and bacteriostatic compound. Industrial Crops and Products 4:23-31.

Pujol, I, Guarro, J, Llop, C, Soler, L, & Fernández-Ballart, J, 1996. A comparison study of broth macrodilution and microdilution antifungal susceptibility tests for the filamentous fungi. *Antimicrobial Agents and Chemotherapeutics*, 40, 2106-2110.

Re, R, Pellegrini, N, Proteggente, A, Pannala, A, Yang, M, & Rice-Evans, C, 1999. Antioxidant activity is applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine,* 26**,**1231-1237.

Ren, W, Qiao, Z, Wang, H, Zhu, L, & Zhang, L, 2003. Flavonoids: Promising Anticancer Agents. *Medicinal Research Reviews*, 23(4), 519 – 534.

Saija, A, Scalese, M, Lanza, M, Marzullo, D, & Bonina, F, 1995. Flavonoids as antioxidant agents: the importance of their interaction with biomembranes. *Free Radical Biology and Medicine*, 19(4): 481-486.

Salvador, MJ, Ferreira, EO, Pral, EMF, Alfieri, SC, Albuquerque, S, Ito, IY, & Dias, DA, 2002. Bioactivity of crude extract and some constituents of *Blutaparon portulacoides* (Amaranthaceae). *Phytomedicine*, 9, 566-571.

Sandoval, M, Charbonnet, RM, Okuhama, NN, Roberts, J, Krenova, Z, Trentacosti, AM, & Miller, MJS, 2000. Cat's claw inhibits TNF alpha production and scavenges free radicals: role in cytoprotection. *Free Radical Biology and Medicine*, 29, 71-78.

Siqueira, JC, 1987. Importância alimentícia e medicinal das Amaranthaceaes do Brasil. *Acta Biologica Leopoldensia,* 9, 5-22.

Sreejayan, N, & Rao, MNA, 1997. Nitric oxide scavenging by curcuminoids. *Journal of Pharmacy and Pharmacology*, 49, 105-107.

Vlietink, AJ, Van Hoof, L, Totte, J, Laure, H, Vanden Berhe, D, Rwangabo, PC, & Mrukiyunmwami, J,

1995. Screening of hundred Rwandese Medical Plants for Antimicrobial and Antiviral Properties. *Journal of Ethano pharmacology*, 46, 31-47.

Wheat, LJ, 1994. Fungal infections in the immunocompromised host. *In:* Rubin, R.H.; Young, L.S. (eds.). Clinical approach to infection in the compromised host. vol.3, (pp.211-237). New York: Plenum Publishing Corporation.

Yildirim, A, Mavi, A, Oktay, M, Kara, A. A, Algur, O.F, & Bilaloglu, V, 2000. Comparison of antioxidant and antimicrobial activities of Tilia (*Tilia argentea* Desf Ex-DC), Sage (*Savia triloba* L.), and Black Tea (*Camellia sinensis*) extracts. *Journal of Agricultural Food Chemistry*, 48(10), 5030-5034.