EFFECT OF Zn, Fe AND Cu CONTENT ON PHYTOCHEMICAL INVESTIGATIONS AND ANTIMICROBIAL POTENTIAL OF *ALTERNANTHERA BRASILIANA*(L.) O. KUNTZE LEAF EXTRACTS PROCURED FROM TWO DIFFERENT STATES OF INDIA

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Abstract

*The effect of zinc (Zn), iron (Fe) and copper (Cu)on phytoconstituent content and the potential antibacterial activity ofthree different leaf extracts of Alternantherabrasiliana(AB) against pathogenic bacteria, procured fromtwo different states of India, was evaluated. Shade dried leaf samples were separately determined for Zn, Fe and Cu content through atomic absorption spectrophotometer (AAS). Furtherextractionwas carried out using aqueous, methanol and mixture of aqueous and methanol (1:1) and microbiosidepotential were evaluated by agar well diffusion technique. Ampicillin (30µg/ml) was used as standard. The Minimum Inhibition Concentration (MIC) of all the extracts was carried out by serial dilution method and thereafter inhibitory microbial activity was carried out by agar plate method. The amount of Zn, Fe and Cu were higher in West Bengal sample. Furthermore, the concentration dependent (**P<0.05) potential antimicrobial activity was observed. At the dose of 200 mg/ml,combined aqueous and methanol extract of AB (ABAE and ABME respectively) from West Bengal, gave significant results against gram positive bacteria where maximum zone of inhibition was recorded against Straphyloccusaureus(13.0± 0.011**).Such variation may be due to the geographical location of the plant, soil pH, content of elements and on theeffects of choice of solvent.*

Key words: Antimicrobial study, agar diffusion technique, elemental analysis, extracts, *Alternantherabrasiliana,* MIC.

Introduction

Plants are much exploited as traditional natural sources for the treatment of various diseases due to the minimum side effects. India is diverse country with sources of huge herbal plants that are used for curing of various diseases traditionally and further documented by researchers with their various methods. These evidences are the future pathway for the development of various formularies and their scientific

datarevealed new drug discovery in global health complications. The medicinal value of plants like antimicrobial, anticancer, anti-inflammatory, antidiabetic, antioxidant, antidiureticetc. mainly depends on the secondary metabolites and the most important bioactive compounds of plants are alkaloids, saponins, flavonoids, tannins, sterols and phenolic compounds(1, 2).Hence all the plants from different location around the world are extracted, purified to investigate their valuable therapeutic activities. The uses of wide range of antibiotics are gradually reduced because of non-affordable cost and also due to microbial resistance (3). Furthermore, plants are reported to have multi medicinal and pharmacological activities so they are the best alternate to overcome microbial drug resistant and therefore researchers are looking forward for discovery of plant based novel drug molecules and formularies. As a result the herbal based industries in India are growing significantly.

Of late, *Alternantherabrasiliana*(AB) (Family: Amaranthaceae) is such deciduous tree, native of South America, is abundantly distributed throughout tropical countries. In India the plant is located in hot areas viz.Andhra Pradesh, Karnataka, Kerala, Madhya Pradesh and West Bengal. Traditionally the leaf is useful in treating cuts, wounds, bruises, ulcers, galactagogue, cholagogue, abortifacient and febrifuge(4).Further pharmacological research and data confirmed that the plant extract exhibited antinociceptive, antimicrobial, analgesic, antiviral activity, wound healing activities (5-8) due to presence of important chemical constituents namely alkaloids, steroids, glycosides, phenols, saponins, flavonoids and terpenes (9). It is well known that any pharmacological activity mainly depends on the active constituents present in the plant and the percentage of the same and the activities are varied with the location and other climatic factors. Further, the plant analysis has been considered as a superior diagnostic technique than soil testing for assessing different elements. Most of the elements are not uniformly distributed in the plant. However to obtain representative plant sample, an attempt should be made to pick up a particular plant part only. But in that case there will be further problems of selecting the appropriate plant parts. Keeping all these problems in view, an attempt was made to select a particular physiologically active leaf for the present elemental analysis.Because of its important traditional uses and easy accessibility in various places of India, suggest that the study of this plant with new thought can be interested for human health and hence the present study was envisaged to evaluate the impact of geographical location of AB in relation to some essential metal content and its relation to phytochemicals and antimicrobial efficacy on selected bacterial stains.

Materials and Methods

Procurement of Plant material

The plant was collected in the month of September, 2013 from Botanical Garden of Kolkata, West Bengal (situated at the longitude of 88º 30'E - 22º 33' N.), and from Rani Channama Agricultural University, Gokak, Karnataka (situated at the longitude of 74° 52' E - 16° 11' N), India (Figure-1). Both the plant were collected in July-August, 2013, further identified and authenticated by Dr. T. N. Shivananda, Principal Scientist, Indian Institute of Horticultural Research, Bangalore, India. The plant specimens were keptindepartment of Pharmacognosy and Phytochemistry as herbarium in Gautham College of Pharmacy with voucher specimen no 231/ Pharmacognosy/ Trivedi/18.

Determination of Zn, Fe and Cu

Pretreated sample with HNO_3 was placed in digestion flask and then mixed with appropriate amount of the ternary acid mixture, consisting of 5 ml for 1gm of powdered plant sample. Digestion was carried out at 180^0C to 200^0C until dense white fumes of H_2SO_4: $HClO_4$ were evolved. The digestion was continued at 180^0C to 200^0C until the acid was largely volatilized and the residues in the flask were clear white and only slightly moist with H_2SO_4. The residue was diluted with glass distilled water and made up to definite volume in a volumetric flask for analysis of Zn, Fe and Cu with the help of Atomic Absorption Spectrophotometer (AAS) Perkin Elmer model Analyst 100.The common oxidant/fuel combination used in AA was air-acetylene. The wavelengths were selected for the analysis based on the concentration ranges of sample and was determined using flame ionization system using Zn at 214 nm, Fe at 372 nm and Cu at 327 nm. The concentration of the above said elements was determined by using the standard condition. The method was based upon the linear relation between the absorbance (AU) and concentration of the determined element. Samples were made in triplicate. The obtained results were expressed in mg/kg on a dry weight in samples.

Preparation of Extracts

The leaf was shade dried and powdered in a mixer grinder. Coarsely powdered samples are stored at room temperature in a sealed plastic covers to avoid microbial contamination due to moisture and used for extraction. Separately about 250 g of the powdered leaf was extracted with aqueous, laboratory grade methanol and with the mixture of equal ratio of aqueous and methanol solvent (1:1) by hot maceration method at 45^0 C. Thereafter all the extracts were filtered with Whatman No. 1 filter paper, evaporated with the rotary flash evaporator at 45°C and stored in refrigeration condition (at 4^0C) in glass bottles for further investigation and furthermore the yield of extracts was calculated.

Phytochemical Screening

All the extracts were qualitatively tested for the identification of various chemical constituents namely alkaloids, flavonoids, steroids, tannins, glycosides and terpenoids as methods described by following standard methods [10, 11].

Phytochemical Screening

Test for Alkaloids: A fraction of extract was treated with 3-5drops of Wagner's reagent and observed for the formation of reddish brown precipitate.

Test for glycosides: Crude extract was mixed with 2ml of chloroform. Then 2ml of concentrated H_2SO_4 was added carefully and shaken gently. A reddish brown color indicated the presence of steroidal ring, i.e., glycone portion of the glycoside.

Test for Carbohydrates: Few drops of Molisch's reagent were added to 2ml portion of the various extracts. This was followed by addition of 2ml of conc. H_2SO_4 down the side of the test tube. The mixture was then allowed to stand for two-three minutes. Formation of a red or dull violet color at the interphase of the two layers was a positive test.

Test for Flavonoids: 2ml of extracts was treated with few drops of 20% sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute hydrochloric acid, indicates the presence of flavonoids.

Test for Phenols: A fraction of the extracts was treated with aqueous 5% ferric chloride and observed for formation of deep blue or black color.

Test for Amino acids and Proteins: 2ml of filtrate was treated with 2-5 drops of ninhydrin solution placed in a boiling water bath for 1-2 minutes and observed for the formation of purple color.

Test for Saponins: In 2ml of extract,few ml of water was added in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins.

Test for Sterols: 1ml of extract was treated with drops of chloroform, acetic anhydride and conc. H_2SO_4 and observed for the formation of dark pink or red color.

Test for Tannins: 2mls of extract was treated with 10% alcoholic ferric chloride solution and observed for formation of blue or greenish color solution.

Test for Terpenoids: 1ml of chloroform was added to 2ml of each extract followed by a few drops of concentrated sulphuric acid. A reddish brown precipitate produced immediately indicated the presence of terpenoids.

Test for Resins: 1ml of various solvent extract were treated with few drops of acetic anhydride solution followed by 1ml of conc. H_2SO_4. Resins give various colorations.

Microorganisms Used

Three gram positive bacterial strains viz. *Bacillus substilis* ATCC 6633, *Straphyloccusaureus* ATCC 29726, *Streptococcuspyogenes* ATCC 13813 and three gram negative bacterial strains namely *Pseudomonasaeruginosa* ATCC 25619, *Escherichia coli*ATCC 8739 and *Serratiamarcescens* ATCC were used as source of microorganismfor the present study [3, 12]procured from department of Microbiology, Bangalore University, Bangalore, India. They were grown and maintained by subculturedon nutrient agar medium in Dept. of Pharmaceutical microbiology, Gautham College of Pharmacy, Bangalore.

Stock solution of Ampicillin as standard was prepared as 30 µg/ml (w/v) concentration in sterile distilled water[13, 14] and 0.1 ml Ampicillin was used as standard for antimicrobial activity in thepresent experiment [3, 15].

Determination of minimum inhibitory concentration (MIC)

Colony made from 24 hour culture of bacterium inoculated to Muller Hinton culture medium (Hi Media Lab, India). 1.0 ml of the extract solution at concentrations of 200 µg/ml was added to 1 ml of Nutrient Broth to obtain extract concentrations of 150, 100, 50 and 25µg/ml for both the separate plants collected from different zones[3, 16]. 0.1ml of each concentration was added to each 9 ml of Nutrient Broth containing standardized test organism of bacterial cells and 0.5 McFarland turbidity standard (1.0 x 10^8cfu/ml) wasinoculated in each test tube [3, 17]by serial dilution method. Thereafter the tubes were thenincubated at 37^oC for 24 hours after incubation; growth was detectedand depends on MIC value the further dose was fixed for the microbioside activity.

Antimicrobial Assay

Antibacterial activity of all the different extracts was assessed by the agar well diffusion method [18] where each isolated microbe was subculture on the recommended specific media for each microorganism at 35-37°C for 25 h. All the extracts (100 mg) were sterilized by filtration through a membrane filter. 6 mm discs were impregnated with sterile cock borer and 50µl of each extract were placedin the wells of agar plates inoculated with microbial culture (after dilution). Ampicillin (30µg/disc) was used as standard and thereafter the plates were incubated at 37^0 C for 16 hours [3] and an antibacterial activity was observed and recorded zone of inhibition (mm) by using sliding calipers from the back of the inverted Petri dishes [19].Triplicate readings were taken to minimize the error.

Statistical analysis:

Analysis of elements was calculated by taking mean values of three replicated set of data with M-Stat software. Further all the microbioside activities were expressed as the mean ± standard error of mean (SEM) where values of $^{***}P<0.001$, $^{**}P < 0.01$and $^{*}P < 0.05$ were considered statistically significant.

Microsoft excel and graph pad prism 5 were used for tabulation of the graphs for MIC and antimicrobial activity determination respectively.

Results and Discussion

Amount of Zn, Fe and Cu

Dried leaf samples after digestion with strong acids, were analyzed for essential micronutrient content (Zn, Fe and Cu) by AAS and the results were showed in Table-1. Results revealed that sample procured from West Bengal showed high content of Fe, Zn and Cu than that of Karnataka sample. This is mainly due to the soil nature. The collected samples were from two different soil zones viz. Kolkata (West Bengal) which is acidic soil and Gokak (Karnataka) which is calcareous soil. Several literature studies reported that calcareous soil content less amount of essential micronutrient [3, 20] as a result our study also showed similar element content. As per the result Fe content was higher in West Bengal soil (5.10 mg/kg) whereas Cu content was lesser (0.56 mg/kg) but the amount were higher than that of Karnataka sample (Fe, 2.58 mg/kg and Cu 0.27 mg/kg).

Yield of extracts

The percentage yields of different extracts procured from two different zones of India, were separately calculated and the results are tabulated in Figure-2. The figure clearly indicated that combined aqueous and methanol extract (1:1) gave more yield in terms of % w/w for both the cultural zone of India, i.e. West Bengal and Karnataka (7.8 and 7.4 % respectively). This higher yield may be due to the soil nature and the content of the major and minor elements present. Interestingly, Cu content was less in both the soil zones which enhanced the content of Fe and Zn that increased the leaf biomass. This result was similar to that of research carried out by Kumar et al., (2009) [21]. Furthermore there was increased amount of extracts procured from the West Bengal sample than Karnataka sample. It was revealed that amount of extract was directly correlated with the leaf biomass and choice of solvent [3, 22, 23]and our result also trend the same as reported earlier in the literature.

Phytochemical study of the extracts

Preliminary phytochemical investigations of different extracts of AB were investigated and revealed the presence of saponins, cardiac glycosides, steroids, tannins and flavonoids as major secondary constituents in both the extracts. The colors of the extracts were wine red, dark red and deep red respectively with the ABAE, ABME and ABAE+ABME (Table-2). This is due to the choice of solvent. In

our study combined equal proportion (1:1) of aqueous and methanol solvents were used which enhanced solubility of the secondary metabolites and showed positive results. The result also correlated with the earlier reports [24, 25].

Determination of minimal inhibitory concentration (MIC)

The results demonstrated the MIC values of all the extracts and resulted inhibition of the strains of all microorganisms was ranges from 10.0 to 230 µg/ml(Figure-3) and 15.0 to 250 µg/ml(Figure-4) for West Bengal and Karnataka samples respectively. Based on the MIC values, further dose were selected for the antimicrobial study.

Antibacterial Assay

All the three different extracts of West Bengal and Karnataka samples were studied for antibacterial activity and resulted significant dose dependent inhibition ($p<0.05$) against all the microorganisms but the results were lesser than that of standard Ampicillin. Combined methanol and aqueous extract (1:1) showed maximum inhibition against gram positive organism (Figure-5 and 6). Sample procured from West Bengal, showed maximum inhibition against all the pathogens than Karnataka sample where maximum zone of inhibition showed against *Straphyloccusaureus*($13.0\pm0.011^{*}$) followed by *Streptococcuspyogenes* ($11.4\pm0.021^{*}$) and lowest activity against *Pseudomonasaeruginosa*($8.0\pm0.005^{*}$) at the concentration of 200 mg/ml. The same resultalso followed by the extracts of Karnataka AB sample, where higher inhibition resulted by combined extract against *Straphyloccusaureus*($12.0\pm0.201^{*}$)followed by *Streptococcuspyogenes*($10.2\pm0.001^{*}$) and lowest activity against *Serratiamarcescens*($8.0\pm0.002^{*}$).

It was observed that the antimicrobial effect of plant extract varies with different factors viz. the effect ofclimate, soil composition, age of plant, quality, quantity, solvent used for extraction, extraction conditions, composition of extracted product and different bacterial strains [26, 27] but the impact of metallic elements on antimicrobial activity has greatly recognized in wide range of pathogens [28, 29].Keeping in view, the present investigation was carried out with determination of micro elements such as Fe, Zn and Cu in raw leaf samples collected from Eastern and Southern zone of India and their impact on pathogens. Hence three different extracts including aqueous, alcohol and combined solvents were used. Interesting antimicrobial activity was resulted which depends on either secondary metabolites extracted by selective solvents or may be the effect of microelements present in the samples. Phytoconstituents like saponins, steroids, tannins and flavonoids havebeen found wide range of biological activities like antimicrobial,anti-inflammatory, analgesic, anti-allergic and antioxidant properties [30] that all were present in the plant resulted in Table-1. Furthermore, the present study focused on an attempt to

investigate the role of Fe, Zn and Cu on potential bactericidal effect. The data provided in the present investigation showed remarkable antibacterial activity against gram positive bacterial which was nearly to the standard Ampicillin. In our study, Cu content was less than Zn and Fe in both the soil zone but was more in acidic soil (West Bengal) which may enhance the antimicrobial activity. The earlier literatures also revealed the similar results where more concentration of Cu showed maximum zone of inhibition against pathogens [31, 32].Literature survey revealed zone of inhibition of AB plant was more in case of Gram positive and Gram negative bacteria but our study revealed less than the reported data [33], this result may be due to higher amount of Fe and Zn content in the AB plant samples that binds with the plant molecules strongly and hence resulted decrease in the antimicrobial potency of both gram positive and gram negative bacteria which also correlated with the earlier literature [32].

Conclusion

Sample procured from West Bengal showed significant concentration dependent potential antimicrobial activity but the activity was less than standard drug Ampicillin. The combined equal mixture of aqueous and methanol leaf extract i.e. (ABAE + ABME) gave high activity against Gram positive pathogens due to the presence of more secondary metabolites and the content of microelements viz. Fe, Zn and Cu. This study confirmed that potent antibacterial activity not only depends on the choice of solvents or any other conditions but also depends on the geographical source and maco and microelements content of the plant that markedly produce pharmacological activities.

Acknowledgements

Author is thankful to Dr. Medhini, Bangalore University for provided microbial strains for the present study. Further extended help to Dr. T. N. Shivananda, IIHR, Bangalore, for authentication of the plant materials and to Prof. Dr. Dilip Kumar Das, HOD, Department of Agriculture and Soil Chemistry, BCKV, West Bengal for carried out elemental analysis by AAS.

References

1. Abdillahi HS, Stafford GI, Finnie JF, Staden JV. Ethnobotany, phytochemistry and pharmacology of *Podocarpussensus*latissimo (S.I). S. Afr. J. Bot 76 (1), 1–24, 2010.
2. Garboui SS, Borg-Karlson AK, Palsson K. Tick repellent properties of three Libyan plants. J. Med. Entomol 46 (6), 1415– 1419, 2009.
3. Das K.Phytochemical evaluation and comparativeantibiocide efficacy of Aqueous, Ethanolic and equalmixture of aqueous and ethanolic (1:1) bark extractof*Lanneacoromandelica*L. procured from Easternregion of India.International Letters of Natural Sciences26,21-31, 2014.
4. Anonymous.The Wealth of India - Raw Materials, Council of Scientific & Industrial Research, New Delhi, pp. 206-207,2005.
5. Lagrota MHC, Wigg MD, Santos MMG, Miranda MMFS. Camara FP, Couceiro JNSS, Costa SS.Inhibitory activity of extracts of*Alternantherabrasiliana* (Amaranthaceae) against the herpes simplex virus. Phytotherapy Res 8, 361, 1994.
6. Coelho de Souza G, Haas APS, Von Poster GL, Schapoval EES, Elisabetsky E. Ethnopharmacological studies of antimicrobial remedies in the south of Brazil. Journal of Ethnopharmacology 90, 135–143, 2004.
7. Barua CC, Talukdar A, Begum SA, Sarma DK, Pathak DC, Borah P.Antinociceptive activity of methanolic extract of leaves of*Alternantherabrasiliana* Kuntz. In animal models of nociception. Pharmacology online 3, 49,2009.
8. Barua CC, Talukdar A, Begum SA, Sarma DK, Pathak DC, Barua AG, Bora RS.Wound healing activity of methanolic extract of leaves of*Alternantherabrasiliana* Kuntz. Using *in vivo* and *in vitro* model. Indian J Exp. Biology 47, 1001, 2009.
9. Harborne JB.Phytochemical methods, in Guide to modern techniques of plant analysis. 2nd edition, (Chapman and Hall, India). pp. 653, 1991.
10. Harborne JB. (eds.). Phytochemical Methods: A guide to modern techniques of plant analysis. 3rd ed. Chapman and Hall, London ISBN: 0-412-57270-2, pp. 302, 1998.
11. Sazada S, Verma A, Rather AA, Jabeen F, Meghvansi MK. Preliminary phytochemicals analysis of some important medicinal and aromatic plants. Adv. in Biol. Res 3, 188-195, 2009.
12. Das K, Gupta NK, Sekeroglu N.Studies on Comparative Antimicrobial Potential of Cultivated Patchouli Oil and Marketed*Eucalyptus* Oil. International Journal of Natural and Engineering Sciences 5 (3), 1-7,2011.
13. Das K, Dang R, Gupta N.Comparative antimicrobial potential of different extracts of leaves of *Stevia rebaudiana* Bert. International Journal of Natural and Engineering Sciences. 3 (1), 65-68.

14. Das, S.K., 2014.Role of Micronutrient in Rice Cultivationand Management Strategy in OrganicAgriculture—A Reappraisal. Agricultural Sciences5, 765-769, 2009.
15. Kaur P, Kumar N. Shivananda TN.Kaur G.Phytochemical screening and antimicrobial activity of the plant extracts of *Mimosa pudica* L. against selected microbes. Journal of Medicinal Plants Research 5(22), 5356-5359, 2011.
16. Atata RF, Sani A, Ajewole SM. Effect of stem bark extracts of *Enantiachloranta* on some clinical isolates. Biokemistri 15(2), 84-92, 2003.
17. Rios JL, Recio MC, Vilar A.Screening methods for natural products with antimicrobial activity: A review of literature. Journal of Ethnopharmacology23, 127-149, 1988.
18. Bauer AW, Kirby WM, Sheriss JC, Turck M.Antibiotic susceptibility testing by a standardized single disk method. Am J ClinPathol45, 493-496, 1966.
19. Kaur R, Jaiswal ML, Jain V.Protective effect of *Lanneacoromandelica*Houtt. Merill. against three common pathogens. J Ayurveda Integr Med 4, 224-228, 2013.
20. Aref F.Manganese, iron and copper contents in leaves ofmaize plants (*Zea mays* L.) grown with different boron and zinc micronutrients. African Journal of Biotechnology 11(4),896-903, 2012.
21. Kumar R, Mehrotra NK, Nautiyal BD, Kumar P, Singh PK. Effect of copper on growth, yield and concentration of Fe, Mn, Zn and Cu in wheat plants (*Triticum aestivum* L.). Journal of Environmental Biology 30(4), 485-488, 2009.
22. Das K, Dang R, Hiremath SR.Effect of cultural conditions on relative sweetness of *Stevia* cultivated under acidic soil zone of South India. International Journal of Agriculture and Food Science 2(3), 108-114, 2012.
23. Dent M, Uzelac VD, Penic M, Brncic M, Bosiljkov T, Levaj B.The Effect of Extraction Solvents, Temperature and Time on the Composition and Mass Fraction of Polyphenols in Dalmatian Wild Sage (*Salvia officinalis*L.) Extracts. Food Technol. Biotechnol 51 (1), 84–91, 2013.
24. Akinsulire R, Aibinu E, Adenipekun T, Adelowotan T, OdugbemiT. In vitro antimicrobial activity of crude extracts from plants *Bryophyllumpinnatum* and *Kalanchoecrenata*. African Journal of Traditional, Complementary and Alternative Medicines 3, 338–344, 2007.
25. Yisa J. Phytochemical analysis and antimicrobial activity of *Scopariadulcis* and *Nymphaea lotus*. Australian Journal of Basic and Applied Sciences 3, 3975–3979, 2009.
26. Bedi N, Bedi PMS, Bodiwala HS, Singh IP, Bansal P. Scientific evaluation of an innovative herbal medicine for relief in respiratory disorders. Canadian journal of pure & applied sciences4(3), 1249-1255, 2010.

27. Bakht J, Tayyab M, Ali H, Islam A, Shafi M. Effect of different solvent extracted sample of *Allium sativum*(Linn) on bacteria and fungi. African Journal of Biotechnology 10(31), 5910-5915, 2011.
28. Sharma S, Ramani J, Bhalodia J, Patel N, Thakkar K, Patel R. Synthesis, Characterization and Antimicrobial Activity of Some Transition Metal Complexes (Mn, Co, Zn, Ni) With L-Proline and Kojic Acid. Adv. Appl. Sci. Res. 2, 374-382, 2011.
29. Osowole AA, Balogun SA. Spectral, Magnetic, Thermal and Antibacterial Properties of Some Metal (II) Complexes of Aminoindanyl Schiff Base. Eur J Appl Sci 4, 6-13, 2012.
30. Shimada T. Salivary proteins as a defense against dietary tannins. J. Chem. Ecol 32(6), 1149-1163, 2006.
31. Otludil B, Aguloglu OB, Demir R, Tolan V, Temel H. The effect of extracellular and membrane in amylase production ofthe tetradentate Schiff base, its Mn(II), Ni(II), Cu(II) and Zn(II) complexes and metal ions in *Bacillus subtilis*. Biotechnology and Biotechnological Equipment 19 (2), 105–110, 2005.
32. Daboor SM, Haroon AM.*In vitro*: Antimicrobial potential and phytochemical screening of some egyptian aquatic plants. Egyptian Journal of Aquatic Research 38, 233–239, 2012.
33. Jalalpure SS, Agrawal N, Patil MB, Chimkode R, TripathiA.Antimicrobial and wound healing activities of leaves of *Alternantherasessilis* Linn. Int J Green Pharm 2, 141-144, 2008.

Figure-1. Collection of plant materials from two different bioclimatic zones of India

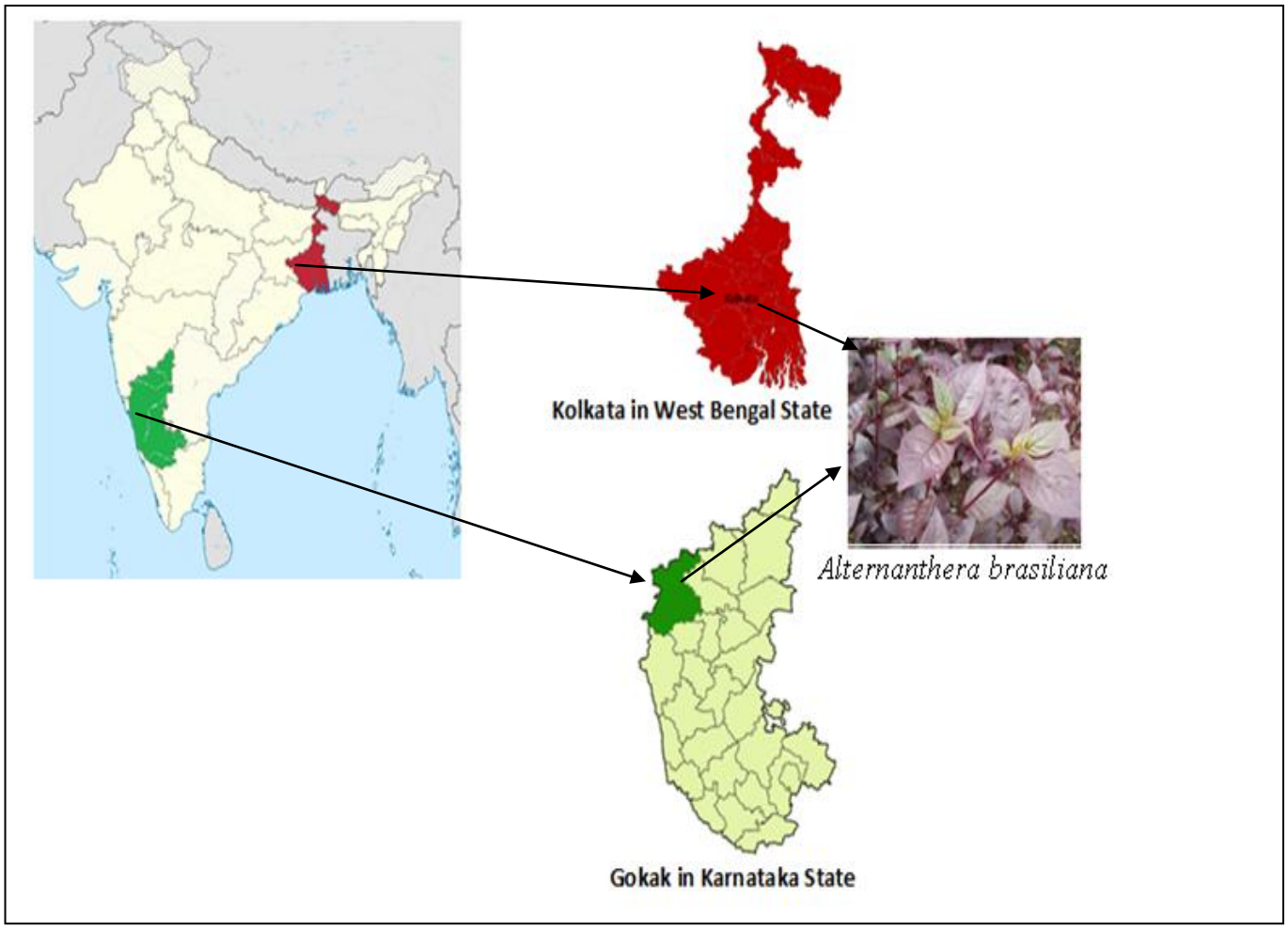
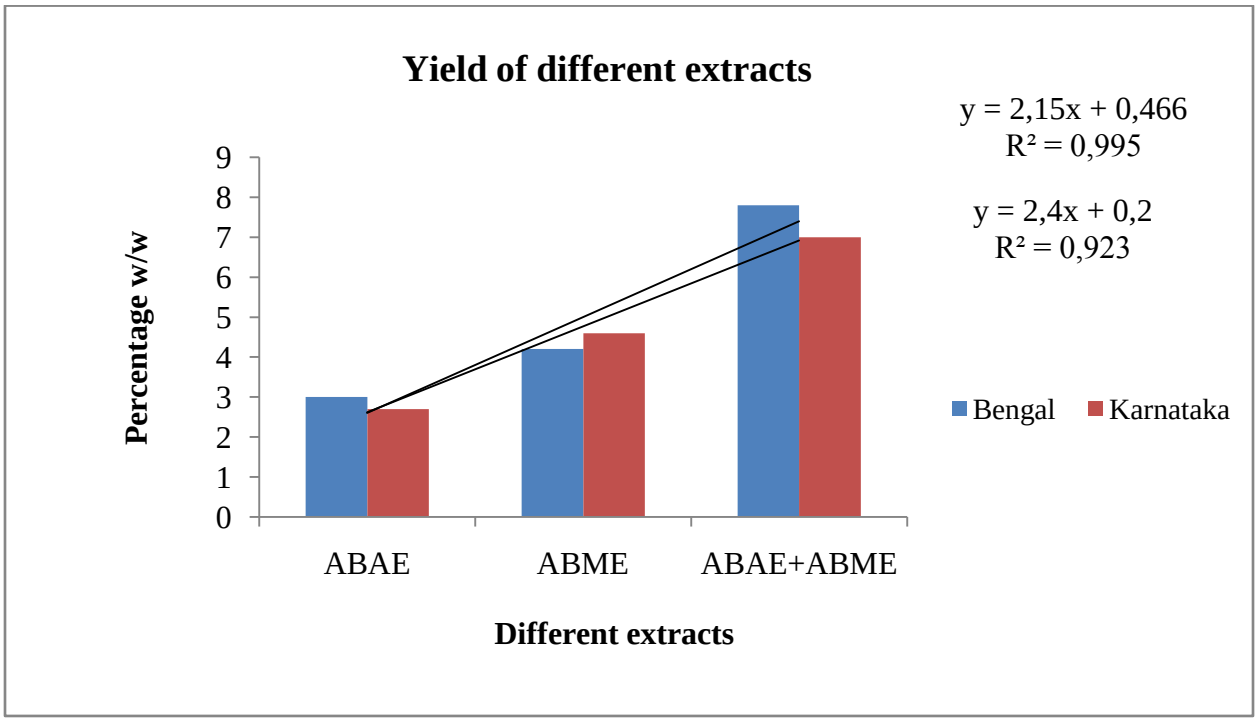
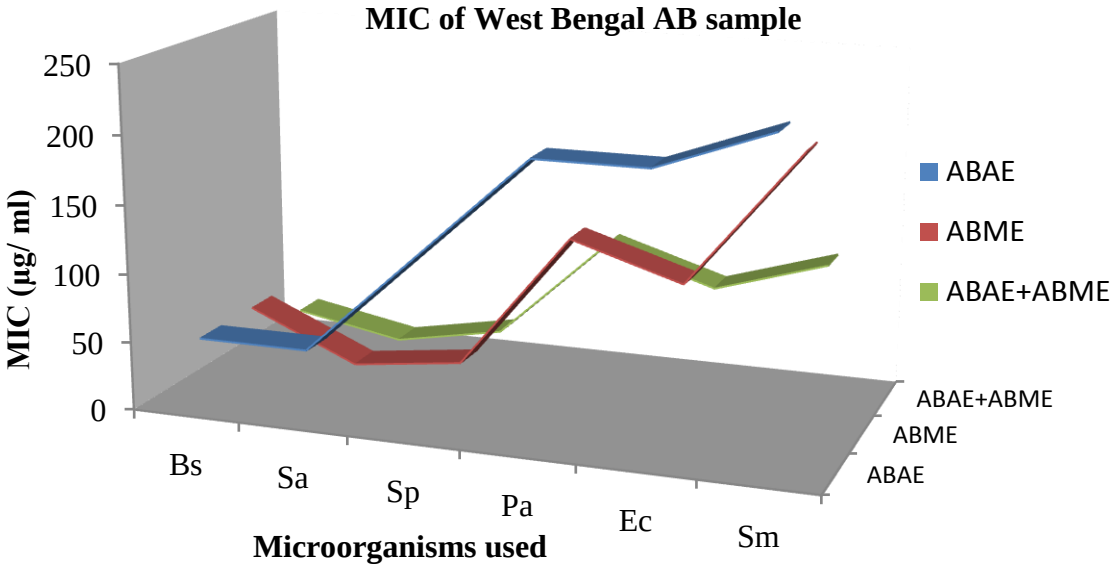


Figure-2.Yield of different extracts procured from two different soil zones of India



- ABAE = *Alternanthera brasiliana* aqueous extract; ABME = *Alternanthera brasiliana* methanol extract

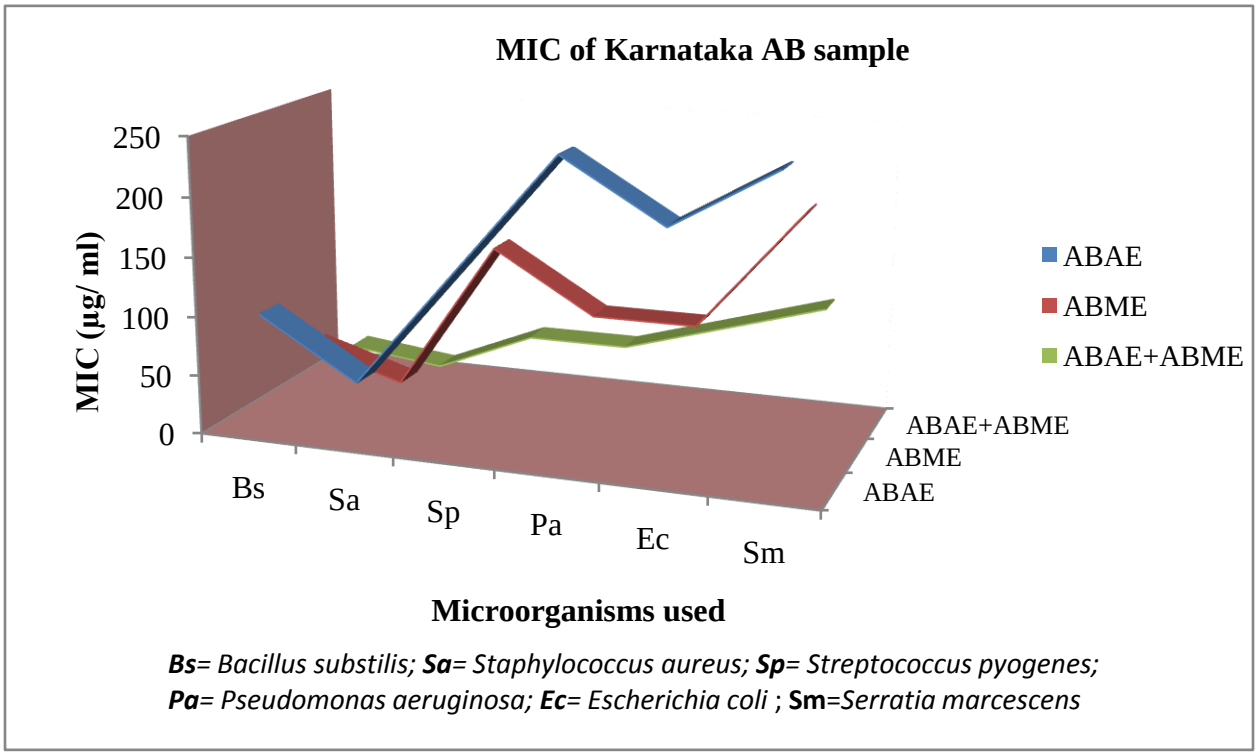
Figure 3: MIC against various microorganisms with all three extracts of AB procured from West Bengal



***Bs**= Bacillus substilis; **Sa**= Staphylococcus aureus; **Sp**= Streptococcus pyogenes; **Pa**= Pseudomonas aeruginosa; **Ec**= Escherichia coli* ; **Sm**=*Serratia marcescens*

- ABAE = *Alternanthera brasiliana* aqueous extract; ABME = *Alternanthera brasiliana* methanol extract

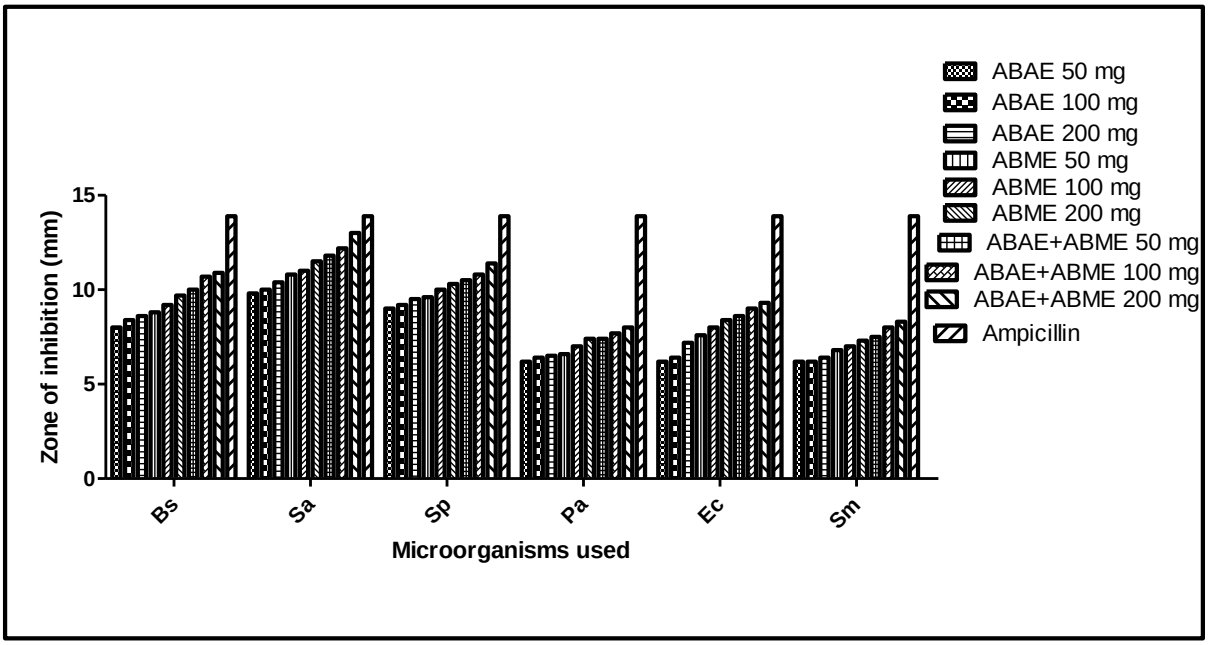
Figure 4: MIC against various microorganisms with all three extracts of AB procured from Karnataka



***Bs**= Bacillus substilis; **Sa**= Staphylococcus aureus; **Sp**= Streptococcus pyogenes; **Pa**= Pseudomonas aeruginosa; **Ec**= Escherichia coli* ; **Sm**=*Serratia marcescens*

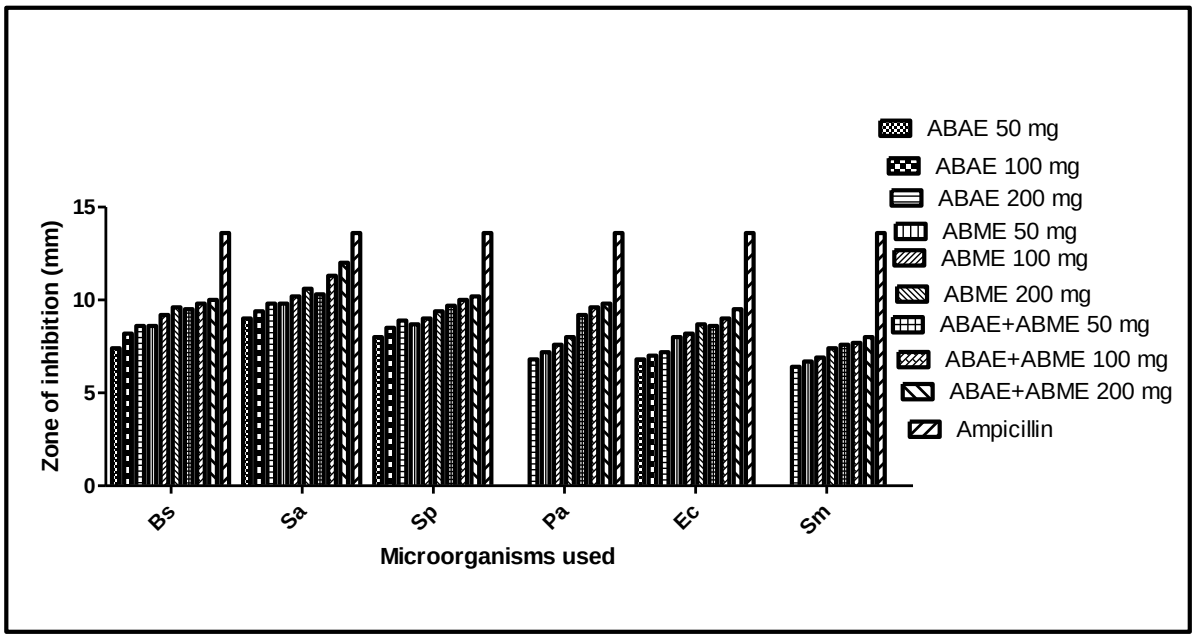
- ABAE = *Alternanthera brasiliana* aqueous extract; ABME = *Alternanthera brasiliana* methanol extract

Figure-5.Antimicrobial study of various extracts of AB procured from West Bengal



- *Bs =Bacillus substilis*; *Sa =Staphylococcus aureus; Sp= Streptococcuspyogenes; Ps= Pseudomonasaeruginosa; Es= Escherichia coli; Sm= Serratiamarcescens*
- ABAE = *Alternanthera brasiliana* aqueous extract; ABME = *Alternanthera brasiliana* methanol extract
- $^{*}P < 0.05$ Significant;

Figure-6.Antimicrobial study of various extracts of AB procured from Karnataka



- *Bs =Bacillus substilis*; *Sa =Staphylococcus aureus; Sp= Streptococcuspyogenes; Ps= Pseudomonusaeruginosa; Es= Escherichia coli; Sm= Serratiamarcescens*
- ABAE = *Alternanthera brasiliana* aqueous extract; ABME = *Alternanthera brasiliana* methanol extract
- $^{*}P < 0.05$ Significant;

Table-1. Elemental analysis of Zn, Fe and Cu for AB collected from two different soil zone of India

States of India	Fe content (mg/kg)	Zn content (mg/kg)	Cu content (mg/kg)
West Bengal	$5.10 \pm 0.002^{***}$	$0.78 \pm 0.013^{***}$	$0.56 \pm 0.012^{***}$
Karnataka	2.58 ± 0.001	0.32 ± 0.011	0.27 ± 0.021
P value:	0.96	0.82	0.82

- Results were triplicated and SEM was calculated.
- $P<0.001^{***}$ = Significant, Preformed unpaired t-test followed by two trials

Table 2: Summary of Comparative analysis of phytoconstituents

Phytoconstituents	West Bengal			Karnataka		
	ABAE	ABME	ABAE + ABME (1:1)	ABAE	ABME	ABAE + ABME (1:1)
Alkaloids	+	+	++	+	+	+
Carbohydrates	+	+	++	+	+	+
Glycosides	+	+	+	+	+	+
Saponins	--	--	--	--	--	--
Phytosterols	--	--	++	--	+	++
Fats & Fixed oils	--	--	--	--	+	--
Resins	--	--	--	--	--	--
Flavonoids	+	++	++	--	++	+
Phenolic acids	--	--	--	--	--	--
Tannins	--	+	+	--	+	+
Proteins	+	--	++	+	+	+
Gums &mucilagenous	--	--	--	--	--	--

(+) = Present in less amount; (++) = Present in high amount; (--) = Absent