Ngule Chrispus Mutuku. et al. / Vol 4 / Issue 3 / 2014 / 163-167.

International Journal of Pharmaceutical Research & Analysis

e-ISSN: 2249 – 7781
Print ISSN: 2249 – 779X

www.ijpra.com

IN VITRO CONTROL OF SELECTED PATHOGENIC ORGANISMS BY METHANOLIC-AQUA EXTRACT OF *ACANTHOSPERMUM AUSTRALE* LEAVES

Ngule Chrispus Mutuku[1], Jackie K Obey[2] and Anthoney Swamy T[1*]

[1]Department of Chemistry, University of Eastern Africa, Baraton, P.O. Box - 2500, Eldoret, Kenya.
[2]Department of Medical Laboratory Sciences, Faculty of MLS, University of Eastern Africa, Baraton, P.O. Box 2500, Eldoret, Kenya.

ABSTRACT

The current study was done to analyse the antibacterial activity of the methanolic- aqua extract of *Acanthospermum australe*. From the study, the plant was found to inhibit only *Bacillus cereus* (20.00± 0.000) among all the other organisms the plant was tested against. Penicillin inhibited the growth of all the organisms while dimethyl sulfoxide (DMSO) did not show any zones of inhibition. The study shows that the plant can be used to treat against infections caused by *Bacillus cereus*, however; more research needs to be done to isolate the active compounds, determine their structural composition and their mode action in inhibiting the bacteria.

Keywords: Acanthospermum, leaves, pharmacological, plants, antibacterial.

INTRODUCTION

The use of medicinal plants is as old as man. Plant extracts have been used since ancient times to treat various diseases affecting human beings. The emergence of drug resistant microorganisms has necessitated the need for alternative drugs to treat against these microorganisms. Scientists' have turned to plants to look for new compounds due to their great potency in the treatment of diseases facing human beings.

In continuation with our interest in the study on medicinal plants [1-6], we take up on *Acanthospermum australe.* The plant *Acanthospermum australe* is an important medicinal plant in the Nandi community in Kenya having a wide range of applications. It is an annual plant in the family Asteraceae. Traditionally plants are used as substitute drugs for various ailments affecting humankind. The information on medicinal value of plants conventionally was passed from generation to generation. This passing of information somehow has led to preservation of the knowledge; however, the trend is changing with many communities abandoning their cultural practices. Since time in immemorial plants have been used as novel source and reservoir of chemical agents with great restorative activities [7-9]. According to Panda [10], nature is a paradise which offers medicinal principles to humanity through plants.

Plants consist of a wide spectrum of compounds which human beings can use in dealing with the ailments affecting them. When God created man He put him in the Garden of Eden and then He planted all kinds of beautiful trees. This marked the beginning of the human kind to use plants as food. The creator had a purpose for providing human beings with plants originally. Christian believe that it is sin which brought sicknesses upon the world and hold to the believe that it is the continued disobedience to the creator which continues to increase the ailments affecting humans and animals today. After man disobedient, God did not provide any alternative source of food and this could impress that the first provision God had given to the human kind was enough to sustain man.

Nature provides us with great source of cheap and safe alternative drugs. According to White [11], the founder of the seventh day Adventist movement, the

Corresponding Author:- T. Anthoney Swamy, Email:- drtanthony2011@yahoo.com

Creator of the universe has given some simple herbs of the field that at times are beneficial and if every society was educated on the use of these medicinal herbs in case of sickness, such suffering might be prevented, and no doctor would be required. These old-fashioned, simple herbs, used intelligently, would have recovered many sick people, who have died under drug medication.

A number of studies have been done to validate the use of medicinal plants in the treatment against microorganisms causing diseases. Studies have shown drug reaction and the side effects they cause have increased the risk of malignancy with fake and adulterated drugs increasing the problem of antibiotic resistance which on the other hand has imposed both biological and economic costs [12-14]. The failure of synthetic drugs to treat against various diseases has led to increased hospitalization and mortality which have been associated with methicillin resistance *Stapylococcus aureus* (MRSA) infections [15].

Medicinal plants have been tested extensively and found to have great pharmacological uses such as anti-inflammatory activity, antibacterial activity, anti- diabetic activity, anti-fungal activity, anti-cancer activity, antioxidant activity, hepatoprotective activity, haemolytic activity, larvicidal activity, anthelmintic activity, pain relief activity, central nervous system activity, sexual impotence and erectile dysfunction and hypolipidemic activity [16-23].

MATERIAL AND METHODS

Sample collection and preparation:

The herb *Acanthospermum austral* was randomly collected in the natural forest around University of Eastern Africa, Baraton in Nandi County. The samples were collected and identified by a taxonomist in the Biology Department, Baraton University. The samples were thoroughly mixed and spread to dry at room temperature in the chemistry laboratory for about three weeks. They were then ground into fine powder and put in transparent polythene bags.

Extraction procedure

Using electric analytical beam balance fifty grams of the powdered leaves of the *Acanthospermum australe*were placed in 1000 ml conical flask, methanol and water were then added in the ratio of 9:1 respectively until the leaves were completely submerged in the solvent. The mixture was then agitated for thorough mixing. The mixture was kept for 24 hours on a shaker for effective extraction of the plant components. The extract was filtered using Butchner funnel; Whatman number 1 filter paper and a vacuum-pressure pump. The filtrate was re-filtered again using the same apparatus. The solvent was evaporated using rotary vacuum evaporator (R -11) with a water bath at 40^{o}C. The extract was brought to dryness using vacuum and pressure pump at room temperature. The residue was then obtained and used for the experiment.

BIOASSAY STUDY

Preparation of the Bacterial Suspension:

The turbidity of each of the bacterial suspension was prepared to match to a 0.5 McFarland standard. The McFarland standard was prepared by dissolving 0.5 g of $BaCl_2$ in 50 ml of water to obtain a 1% solution of Barium chloride (w/v). This was mixed with 99.5 ml of 1% Sulphuric acid solution. Three – five identical colonies of each bacterium was taken from a blood agar plate (Himedia) culture and dropped in Mueller Hinton broth (Himedia). The broth culture was incubated at 37^{0}C for 2 - 6 hours until it achieved turbidity similar to the 0.5 McFarland standards. The culture that exceeded the 0.5 McFarland standard were each adjusted with the aid of a UV spectrophotometer to $0.132A^{0}$ at a wavelength of 600 nm in order to obtain an approximate cell density of $1x10^{8}$ CFU/ml.

Preparation of the Extract Concentrations and Antibiotic

Stock solutions for the extracts were prepared by dissolving 500 mg in 1 ml of dimethyl sulfoxide (DMSO). An antibiotic control was made by dissolving 500 mg of penicillin in 1 ml of sterile distilled water. DMSO served as a negative control.

Determination of bioactivity of the Extract

Mueller Hinton agar plates were prepared by the manufacturer's instruction. 1 ml of each of the prepared bacterial suspension for the test was transferred to 2 plates for each organism to give a duplicate for each concentration and organism. Five wells were drilled in each agar plate. Three of the wells were filled with the extract dilution and the other wells were filled with penicillin and DMSO control respectively. The wells were labeled on the underside of the plate. The plates were incubated at 37^{0}C for between 24 to 48 hours and the zones of inhibition were measured in millimeters with the aid of a ruler.

RESULTS AND DISCUSSION

Antibacterial activity (Table 1) showed that only organism inhibited by *A. australe* extract was *Bacillus cereus*.Noinhibition was observed by all the other organisms.

The penicillin control inhibited all the organisms while the DMSO negative control had no inhibitory effect on the organisms.

From the table 2, the zones of inhibition of *A. australe* extract on *B. cereus* was significantly higher than that of *E. coli*, *Salmonella typhi*, *S. liquefaciens*, *Enterobacter aerogenes* and *Proteus vulgaris.* The penicillin control inhibited all the organisms' significantly higher than the extract. The rest of the organisms, when compared pair wise, did not differ significantly in their zones of inhibition by the extract.

From the data obtained in this research, the plants methanolic- aqua extract can be used to treat against the ailments caused by *Bacillus cereus* viz posttraumatic wounds, self-limited gastroenteritis, burns, surgical wounds infections, ocular infections such as endophthalmitis, corneal abscess and panophthalmitis [24&25]. The current study is in conformity with the previous studies in which *Acanthospermum hispidum* aqueous extract was found to have no antibacterial activity against the microorganisms it was tested against. However, the study is different since *Acanthospermum hispidum* was found in other studies to have antibacterial activity against bacterial organisms such as *Enterococcus faecalis* and *Staphylococcus aureus* [26]. According to Fleischer [27], a similar species *A. hispidum* had antibacterial activity against a range of gram positive and gram negative bacteria. The difference in the studies might be due to difference in the solvents used, geographical location and also the period or season at which the samples were collected since these are factors which affect the concentration of the active compounds to be extracted from the plant. The inhibition of the plant against *Bacillus cereus* is noteworthy since the bacterium has been found to be resistant to the currently used antibiotics [28,29].

Table 1. Antimicrobial activity (Mean Zone of Inhibition ± S.E.) of *Acanthospermum austral* against selected microbial organisms

Microorganisms	Mean ±S.E	Penicillin	DMSO
Escherishia coli	0.00±0.00	41.00± o.557	0.00±0.000
Salamanella typhi	0.00±0.00	36.33±0.882	0.00±0.000
Serratia liquefaciens	0.00±0.00	43.67±0.662	0.00±0.000
Entenbacter aerogenes	0.00±0.00	42.00±1.528	0.00±0.000
Bacillus cereus	20.00±0.00	40.67±0.333	0.00±0.000
Proteus vulgaris	0.00±0.00	35.00±1.000	0.00±0.000

Key: S.E. = standard error

Table 2. Tukey's honestly significant Difference among microorganisms using 500 mg/ml of *Acanthosperm australe* leaf extract

Comparison	P-value	Significance
E.coli vs *Salmonella typhi*	1.00	NS
E.coli vs S. *liquefaciens*	1.00	NS
E.coli vs *E. aerogenes*	1.00	NS
E.coli vs *B. cereus*	0.00	S
E.coli vs *P. vulgaris*	1.00	NS
E.coli vs *E.coli* control	0.00	S
Salmonella typhi vs *S. liquefaciens*	1.00	NS
Salmonella typhi vs *E. aerogenes*	1.00	NS
Salmonella typhi vs *B. cereus*	0.00	S
Salmonella typhi vs*P. vulgaris*	1.00	NS
Salmonella typhi vs *S. typhi* control	0.00	S
S. liquefaciens vs *E. aerogenes*	1.00	NS
S. liquefaciens vs *B. cereus*	0.00	S
S. liquefaciens vs *P. vulgaris*	1.00	NS
S. liquefaciens vs S. *liquefaciens* control	0.00	S
E. aerogenes vs *B. cereus*	0.00	S
E. aerogenes vs *P. vulgaris*	1.00	NS
E. aerogenes vs *E. aerogenes* control	0.00	S
B. cereus vs *P. vulgaris*	0.00	S
B. cereus vs *B. cereus* control	0.00	S
P. vulgaris vs *P. vulgaris* control	0.00	S

CONCLUSION

The antibacterial activity of the plant is attributed to the presence phytochemicals in the plant. According to Anthoney [30], the plant leaves were found to contain tannins, saponins, terpenoids, phenols, alkaloids and steroids rings. The previous studies have shown these compounds to have great pharmacological activity, however, the low activity of the plant leaves may be due low concentration of the active compounds due to its geographical location or the seasons in which the plant was collected, since similar species *Acanthospermum hispidum*

showed antibacterial activity against all the bacteria it was tested against.

Research needs to be done using other solvents and also extract the plant compounds in the traditional way. Further research needs to be done to isolate the active ingredients, determine their structural formula, their mode of action and their effect in the in vivo environment.

ACKNOWLEDGEMENT

The authors of this paper are very much thankful to the Department of Chemistry and Department of Medical Laboratory Science, University of Eastern Africa, Baraton for creating space and chemicals to contact this study. We are also thankful to the taxonomist Mr. Joel Ochieng Ondiek for his great assistance in the identification of the plant.

REFERENCES

1. Anthoney ST, Jackie O, Ngule CM. In vitro control of selected pathogenic organisms by *Vernoniaadoensis roots.International Journal of Pharmacy and Life Sciences*, 4(8), 2013, 2855-2859.
2. Anthoney ST, Ngule CM and Machoka R. Histopathological effects of *Vernonia adoensis* roots extract on the larvae of the mosquito *Culex quinquefanciatus*. *Journal of Pharmaceutical Biology*, 4(1), 2014, 1-3.
3. Ngule CM, Anthoney ST and Machoka R. Evaluation of larvicidal activity of *Vernonia adoensis* leaves against *Culex quinquefanciatus*. *International Journal of Phytotherapy*, 4(1), 2014, 11-13.
4. Anthoney ST, Obey JK and Ngule CM. In vitro Antibacterial Activity of Methanolic–Aqua Extract of *Plectranthus agentatus* Leaves. *World Journal of Pharmaceutical Research (WJPR)*, 3(1), 2014, 339-349.
5. Akumu EO, Kebenei S, Anthoney ST and Ngule CM. Repellency of *Lantana camara* Leaves Smoke against Female Anopheles Mosquitoes. *Int J Bioassays*, 3(1), 2014, 1695-1698.
6. Anthoney ST, Ngule CM and Obey J. In Vitro Antibacterial activity of Methanolic-aqua extract of *Tragia brevipes* Leaves. *Int J of Pharm Life Sci*, 5(2), 2014, 3289-3294.
7. Gobalakrishnan R, Kulandaivalu M, Bhuvaneswari R, Kandavel D and Kannan L. Screening of wild plant species for antibacterial activity and phytochemical analysis of *Tragia involucrate* L. *Journal of Pharmaceutical Analysis,* 7(1), 2013, 01-06.
8. Mensah JK, Okoli RI et al. phytochemical analysis of medicinal plants used for the management of hypertension by Esan people of Edo state, Nigeria. *Ethnabot Leaflets*, 13, 2009, 1273-1287.
9. Sandigawad AM. In vitro evaluation of antibacterial activity of bark and flower extracts of *Pimenta officinalis* Lindi. *Adv Biores*, 1(2), 2010, 61-68.
10. Panda D, Dash KS and Dash GK. Phytochemical examination and antibacterial activity of various solvent extracts and the selected isolated compounds from roots of *Tragia involucrata* Linn. *International Journal of Pharmaceutical Sciences and Drug Research*, 4(1), 2012, 44-48.
11. White EG. Medicinal herbs. Retrieved from http//csda-adventistchurch.to/EGW_Messenger.html on 03-04-2014.
12. Chabot S, Bel-Rhlid R, Chenevert R and Piche Y. Hyphal growth promotion in vitro of the VA *Mycorrhizad fungus*, *Gigaspora margarita* Becker and Hall, by the activity of structurally specific flavonoid compounds under CO2 – enriched conditions. *New Phytology*, 12, 1992, 461-467.
13. Chessin M, Deborde D and Zip FA. Antiviral proteins in higher plants. CRC Pres, Inc., Bocaraton, Fla, 1995.
14. Green BO. Significance and efficacy of medicinal plants in the Niger Delta. *Continental Journal of Pharmaceutical Sciences*, 1(3), 2007, 23-29.
15. Arekemase MO, Badandoko AM, Ojo KRM, Elizabeth AA and Kamoldeen AA. Antimicrobial effects of *Garcinia kola* (bitter kola) on some selected pathogens from University of Ilorin teaching hospital Ilorin, Nigeria. *Asian Economic and Social Society*, 2(4), 2012, 157-169.
16. Hosahally RV, Sero G, Sutar PS, Joshi VG, Sutar KP and Karigar AA. Phytochemical and pharmacological evaluation of *Tragiacannabina* for anti-inflammatory activity. *International current Pharmaceutical Journal*, 1(8), 2012, 213-216.
17. Farook SM and Atlee WC. Antidiabetic and hypolipidemic potential of *Tragia involucrata* Linn. In streptozotocin-nicotinamide induced type II diabetic rats. *International Journal of Pharmacy and Pharmaceutical Sciences,* 3(4), 2011, 103-109.
18. Kisangau DP, Hosea KM, Joseph CC and Lyaruu HVM. In vitro anti-microbial assay of plants used in traditional medicine in Bukoba rural district, Tanzania. Afr. J. Traditional, *Complementary and Alternative Medicines*, 4(4), 2007, 510-523.
19. Kamatenesi-Mugisha M and Oryem-Origa H. Traditional herbal remedies used in the management of sexual impotence and erectile dysfunction in western Uganda. *African Health Sciences,* 5(1), 2005, 40-49.
20. Adu F, Gbedema SY, Akanwariwiak WG, Annan K and Boamah VE. The effects of *Acanthospumum hispidum* extract on the antibacterial activity of amoxillin and ciprofloxacin, 3(1), 2011, 58-63.
21. Deeepa N and Rajendran NN. Antibacterial and anti-fungal activities of various extracts of *Acanthospermum hispidum* DC. *Journal of Natural Remedies*, 7(2), 2007, 225-228.

22. Joshi CG, Gopal M and Byregowda SM. Cytotoxic activity of *Tragia involucrata* Linn extracts. *American-Eurasian Journal of Toxicology Sciences*, 3(2), 2011, 67-69.
23. Arivoli S and Tennyson S. Larvicidal efficacy of *Strychnos nuxvomica* Linn. (Loganiaceae) leaf extracts against the filarial vector *Culex quinquefanciatus* Say (Diptera,Culicidae). *World Journal of Zoology*, 7(1), 2012, 06-11.
24. Sankararaman S and Velayuthan S. Bacillus cereus. *Pediatrics in Review*, 34, 2013, 196.
25. Garcia-Arribas ML, Plaza CJ, De La, Rose MC and Mosso MA. Characterization of *Bacillus cereus* strains isolated from drugs and evaluation of their toxins. *J Appl Bacterial*, 64, 1988, 257-264.
26. Cartagena E, Bardon, A, Catgalan AN. Germacranolides and a new type of guaianolide from *Acanthospermum hispidum*. *J Nat Prod*, 63, 2000, 1323-1328.
27. Fleischera TC, Ameadea EPK, Sawerb IK. Antimicrobial activity of the leaves and flowering tops of *Acanthospermum hispidum*. *Fitoterapia*, 74, 2003, 130–132.
28. Sidambiwe JB, Calomme M, Cos P, Totte J, Vlietinck A, Vanden BDA. Screening of seven selected Rwandan medicinal plants for Antimicrobial and antiviral activities. *J Ethnopharmacol*, 65, 1999, 71–72.
29. Mathekga ADM, Meyer JJM, Horn MM, Drews SE. An acylated phloroglucinol with Antimicrobial properties from *Helichrysum caespititum. Phytochemistry*, 53, 2000, 93–96.
30. Anthoney ST, Ngule CM, Obey J. Preliminary phytochemical screening of methanolic-aqua extract of *Acanthospermum austral* leaves. *International Journal of Bioassays*, 2(11), 2013, 1434-1439.