Baraton Interdisciplinary Research Journal (2015), 5(Special Issue), pp 125-132

ASSESSMENT OF ANTIBACTERIAL ACTIVITY OF HOT AQUA EXTRACT OF SENNA DIDYMOBOTRYA LEAVES

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Abstract

Nature is a paradise of medicinal solutions to all ailments affecting human beings through medicinal plants. Medicinal plants are being used widely to treat against the currently widespread strains of drug resistant bacteria. Scientists all over the world are working hard to provide scientific justification on the traditional use of medicinal plants to treat almost all the ailments affecting human beings. Green medicine has attracted great interest due to the belief that it is safe, cheap and more dependable than aliphatic drugs, which have adverse side effects. *Senna didymobotrya* is widely used traditionally to treat against various illnesses such as abdominal pains, stomach problems and as an anthelmintic. The current study was done to investigate the antibacterial activity of infused Senna leaves. From the study, the plant was found to inhibit the growth of all the gram positive and gram-negative bacteria it was tested against. The plant extract inhibited the growth of *Staphylococcus aureus* with a zone of inhibition of 11.00±0.577, *Salmonella typhi* 9.67±0.333, *Proteus vulgaris* 13.00±0.577, *Escherichia coli* 18.33±0.882, *Enterobacter aerogenes* 10.33±0.667, *Bacillus cereus* 14.33±0.333. The data obtained clearly shows how great potency the plant has against bacterial organisms. The results obtained in this research work are a scientific justification on the traditional use of *Senna didymobotrya* to treat against diseases cause by all the bacterial organisms it was tested against.

Keywords: *Senna didymobotrya,* infused, antibacterial, medicine, plants.

Introduction

Plants have been known since ancient times to treat various diseases and therefore scientists have found them to be a better choice in such for bioactive compounds (Khan, Ahmed, Mir, Shukla, & Khan, 2011; Jeyaseelan, Pathmanathan, & Jeyadevan, 2010). In continuation with our research on medicinal plants (Anthoney, Ngule, & Obey, 2013, 2014), we have turned our attention to the leaves of *Senna didymobotrya* plant. It is mainly found along lakeshores, streams, rivers, deciduous, bush land and old plantations. The plant is hardly attacked by diseases or pests. *Senna didymobotrya* is locally known as senetwet. It is used in the preparation and preservation of 'mursik' which is the local name for fermented milk, hence, the name mursik plant (Tabuti, 2007; Ngule, Anthoney, & Obey, 2013). Microbial resistance to the currently used antibiotics has greatly increased in the last four decades despite efforts by pharmaceutical industries to produce new antibiotics. Several measures have been put in place in various countries all over the world to control the spreading of drug resistant microorganisms, however, the microorganisms have continued to develop new ways to mutate and acquire resistance to drugs (Nasciment, 2000). According to Montellia and Levy (1991), data collected on resistant microorganisms shows the period between 1980-1990 to have recorded the highest number of microbial drug resistance. The increase on the number of drug resistance microorganisms calls for quick action to control the situation.

Plants have been used since time immemorial to treat most of the diseases affecting human kind. The introduction of synthetic drugs, however, changed the trend and attracted many to turn to use them on the expense of botanical drugs, a trend which according to researchers is changing and many people are using medicinal herbs. According to Anthoney (2013), about 80% of the individuals from developing countries are using traditionally known plants as medicine. The world health organization (WHO), recommends medicinal plants to be the best source of a variety of drugs (Santos, 1990). Botanical medicine is the oldest known type of medicine. The use of plants as source of medicine is as old as the origin of

man himself. Medicinal plants have been used widely over all the cultures as a source of drugs for treatment of various ailments affecting human beings and animals (Sigh and Singh, 2010). The medicinal values of plants are attributed to pharmacologically active compounds that have no direct impact on the plants main processes but research has proved these compounds to have great medicinal values. These compounds that the plant uses to protect itself against predators are called secondary metabolites or phytochemicals.

Over the recent decades scientist have developed great interest on botanicals to isolate these compounds through various methods such as column chromatography and thin layer chromatography in order to purify them and study their structural elucidation. The studies already done have shown plants to have great potentials in the treatment against drug resistant microorganisms (Muroi & Kuba, 1996).

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, anticancer activity, antioxidant activity, hepatoprotective activity, haemolytic activity, larvicidal activity, anthelmintic activity, pain relief activity, central nervous system activity, sexual impotence and erectile dysfunction (Hosahally, 2012; Farook, 2011; Kisangau, 2007; Kamatenesi, 2005; Adu, 2011; Deepa, 2007; Joshi, 2012; Arivoli, 2012). The plant is used traditionally in the treatment of enteric problems, as an anthelmintic, treatment against fungal infections and in the preservation of milk by the Nandi community in Kenya. The great potency which the plant has demonstrated traditionally therefore creates the need for scientific justification on the medicinal value of the plant. The current study was done to analyse the antibacterial activity of the plant against selected pathogenic microorganisms.

Materials and Methods

Sample Collection and Preparation

The herb was randomly collected in the natural forest around University of Eastern Africa, Baraton. The plant samples were identified by a taxonomist in the University of Eastern Africa, Baraton. 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 leaves of *Senna didymobotrya* was put in a conical flask and heated to boiling for 20 minutes. The extract was filtered using Butchner funnel; Whatman no.1 filter paper , a vacuum and 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 50oC. 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, a procedure similar to that used by Biruhalem (2011) and Donay (2007). The McFarland standard was prepared by dissolving 0.5 g of BaCl2 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 370C 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 0.132A0 at a wavelength of 600 nm in order to obtain an approximate cell density of 1x108 CFU/ml.

Preparation of the extract concentrations and antibiotic. Extracts stoke solutions were prepared by dissolving 500 mg in 1 ml of dimethylsulfoxide (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. 0.1 ml of each of the prepared bacterial suspension for the test was transferred to 3 plates for each organism to give a triplicate 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. Three plates were made for each bacterial organism and extract giving a triplicate reading for each microorganism and extract. The plates were labeled on the underside and incubated at 37^{0}C for between 24 to 48 hours and the zones of inhibition measured in millimeters with the aid of a ruler.

Table 1

Antimicrobial Activity (Mean Zone of Inhibition ± S.E.) of Senna Didymobotrya Leaves Crude Extract

Microorganisms	Mean ± S.E	Penicillin	DMSO
Escherichia coli	18.33 ± 0.882	44.00± 0.577	0.00±0.000
Salmonella typhi	9.66 ± 0.333	30.67 ±0.333	0.00±0.000
Staphylococcus aureus	11.00 ± 0.577	30.33± 0.333	0.00±0.000
Enterobacter aerogenes	10.33± 0.577	39.00±0.000	0.00±0.000
Bacillus cereus	14.33 ±0.333	18.33±0.333	0.00±0.000
Proteus vulgaris	13.00 ±0.577	24.00±0.577	0.00±0.000

From the study, the plant leaves were found to inhibit all the bacterial organisms it was tested against with Escherichia coli having the highest zone of inhibition (18.33 ± 0.882), followed by *Bacillus cereus* (14.33 ±0.333), *Proteus vulgaris* (13.00 ±0.577), *Staphylococcus aureus* (11.00 ± 0.577), *Enterobacter aerogenes* (10.33± 0.577), and *Salmonella typhi* (9.66 ± 0.333), the plant produced the lowest zone of inhibition while the negative control (DMSO) did not show any zones of inhibition. The positive control (penicillin) inhibited the growth of all the organisms it was tested against. The comparison of the zones of inhibition among the microorganisms showed that there was significant difference between the zones of inhibition.

Table 2

Tukey's Honestly Significant Difference Among Microorganisms Using 500 mg/ml of Senna Didymobotrya Leaves Extract

Comparison	P- value	Significance
Staphylococcus aureus vs *Salmonella typhi*	0.775	NS
Staphylococcus aureus vs *Proteus vulgaris*	0.250	NS
Staphylococcus aureus vs *Escherichia coli*	0.000	S
Staphylococcus aureus vs *Enterobacter aerogenes*	0.998	NS
Staphylococcus aureus vs *Bacillus cereus*	0.005	S
Staphylococcus aureus vs *Staphylococcus aureus* control	0.000	S
Salmonella typhi vs *Proteus vulgaris*	0.005	S
Salmonella typhi vs *Escherichia coli*	0.000	S
Salmonella typhi vs *Enterobacter aerogenes*	0.998	NS
Salmonella typhi vs *Bacillus cereus*	0.000	S
Salmonella typhi vs *Salmonella typhi* control	0.000	S
Proteus vulgaris vs *Escherichia coli*	0.000	S
Proteus vulgaris vs *Enterobacter aerogenes*	0.040	S
Proteus vulgaris vs *Bacillus cereus*	0.775	NS
Proteus vulgaris vs *Proteus vulgaris* control	0.000	S
Escherichia coli vs *Enterobacter aerogenes*	0.000	S
Escherichia coli vs *Bacillus cereus*	0.001	S
Escherichia coli vs *Escherichia coli* control	0.000	S
Enterobacter aerogenes vs *Bacillus cereus*	0.001	S
Enterobacter aerogenes vs *Enterobacter aerogenes* control	0.000	S
Bacillus cereus vs *Bacillus cereus* control	0.000	S

Further comparison on the zones of inhibition using the Tukey's pairwise comparison showed that the zones of inhibition of Staphylococcus aureus were significantly lower than those of *Escherichia coli* and *Bacillus cereus* ($p< 0.05$). However, the *Staphylococcus aureus* zones of inhibition were not significantly different from those of the organisms ($p>0.05$). The zones of inhibition the positive control against *Staphylococcus aureus* were significantly higher than those caused by the plant extract against the bacteria ($p<0.05$). *Salmonella typhi* inhibition zones were significantly lower than those of *Proteus vulgaris* ($p<0.05$), *Escherichia coli* and *Bacillus cereus* ($p< 0.001$); however the zones of inhibition of Enterobacter aerogenes were signicant as compared to those of *Salmonella typhi* ($p>0.05$). On the other hand the zones of inhibition of Proteus vulgaris were significantly lower as compared to those of *Escherichia coli* ($p<0.001$) but significantly higher than those of *Enterobacter aerogenes* ($p< 0.05$); however they were not significant as compared to those of *Bacillus cereus*. The zones of inhibition caused by *Escherichia coli* were significantly higher than those of *Enterobacter aerogenes* and *Bacillus cereus* ($p<0.001$). The inhibition zones caused by the plant against Enterobacter aerogenes were significantly lower than those of Bacillus cereus ($p<0.001$).

The results obtained in this research are inconformity with those obtained by Ngule [17], in which the plant leaves were found to inhibit the growth Salmonella typhi with 12.50±0.563, Klebsiella sp., 14.33±0.211, *Bacillus cereus* 19.00±0.258, Streptococcus pyogenes 11.67±0.494, *Escherichia coli 12*.17±0.477, *Proteus vulgaris* 10.83±0.477, *Enterobacter aerogenes* 10.33±0.615. The data recorded is also in conformity with that recorded by Nyaberi (2013), in which the stem charcoal of the plant inhibited the growth of *E.coli* (15.3±0.6) and *P.auroginosa* (13.6±0.5). The study is also in conformity with the previous study contacted by Anthoney (2013) in which the aqueous extract was found to contain the highest percentage of the phytochemicals viz tannins, saponins, terpenoids, flavonoids, alkaloids and steroidal rings were detected. The present

study different and in contrast with previous studies which discredited the use of water as good solvent in the extraction of active compounds from plants (Romero, 2011; Iqbal, 2009; Iqbal, 2012). In this study therefore we affirm the use of traditional method of extraction by using decoction method to extract plant active compounds as earlier stated by Anthoney (2014). The study According to Jeyaseelan (2010), plant extracts may act by interfering with peptidoglycan bacterial cell wall synthesis. They may also inhibit protein synthesis, interfere with nucleic acid synthesis, breaking the peptide bonds, preventing the utilization of available nutrients, lysis of microbial cells and acting as chelating agents inhibiting metabolic pathway (Gobalakrishnan, 2013).

The plant extract can be used to treat infections caused by *Bacillus cereus* viz posttraumatic wounds, self-limited gastroenteritis, burns, surgical wounds infections, ocular infections such as endophthalmitis, corneal abscess and panophthalmitis (Garcia, 1988; Sankararaman, 2013). The plant extract can be used to treat immunologically compromised patients including AIDS and malignant disease victims (Cotton, 1987; Tuazon, 1979). The plant's ability to inhibit the growth of *E. coli* is a scientific justification that the plant can be used treat against enteric infections caused by the bacteria. The plants extract can also be used to treat against gastro-intestinal diseases, ear infections, urinary tract infections and wounds infections caused by *Proteus vulgaris* (Goodwin, 1971; Neter, 1943). Salmonella sp. makes one of the most common food poisoning forms all over the world (Baker, 2007). The data obtained shows that the plant leaves extracted in the simple traditional way can be used to treat against food poisoning caused by *Salmonella typhi*. The plant can also be used to treat against typhoid, paratyphoid fever, traveler's, diarrhea, gastroenteritis in adults and gastroenteritis in children (Hallstrom, 2011). *Senna didymobotrya* can be good source of active compounds for a variety of various diseases affecting human beings in world today.

Conclusion

The result from this study shows that the plant leaves have great pharmacological value against all the organisms it was tested against. The results shows that the compounds from the plant can be extracted with water hence eliminating the use of chemical solvents and in the end solving the problem of pollution associated with these solvents. The plants antibacterial activity is attributed to the presence of important pharmacological compounds in the plant. From the study it is also worthy to mention that the antibacterial activity of the plant could be due to synergistic effect of two or more compounds in the plant. The data obtained in this research is a scientific justification of the plant leaves use in the treatment of various diseases affecting human beings. It is, therefore, worthy to recommend the plant for the treatment of all diseases caused by all the organisms the plant was tested against. Further research needs to be done to isolate the active compounds and analyse their structural composition, their mode action and their effect in the in vivo environment.

Acknowledgements

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 conduct this study. We are also thankful to Mr. Joel Ochieng Ondiek for his great assistance in the identification of the plant.

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