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In Vitro control of selected pathogenic organisms by *Vernonia adoensis* roots

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Abstract

The main aim of conducting this study was to evaluate antibacterial activity of methanol–water extract of *Vernonia adoensis* roots against *Salmonella typi, Klebsiella sp, Bacillus cereus, Streptococcus pyogenes, E. coli, Proteus vulgaris* and *Enterobacter aerogenes*.The roots powder of the plant were extracted with methanol and water in the ratio 9:1. The antibacterial activity of the extract was determined by agar well diffusion method. Vernonia adeonsis roots extract was found to control the growth of only *B. cereus, S. pyogenes* and *Proteus vulgaris* at zone of inhibition of 10.50 ± 0.563, 15.67 ± 0.422 and 11.00 ± 0.577 respectively. From the study methanol- water extract can be used to control B. *cereus, S. pyogenes* and *Proteus vulgaris*. More research needs to be done to identify the specific bioactive compounds, their structural formulas, their mode of action and toxicity in vivo conditions.

Key-Words: *Vernonia adoensis,* Antibacterial activity, Extract, Pathogenic organisms

Introduction

Medicinal plants have been used since ancient times to treat many illnesses. According to Cousins (2002) over 80% of the plants in Nigeria used for treatment of malaria and other sicknesses are also used as food, there seem to be not much distinction between medicinal benefits of plants and their nutritive value.

The published WHO traditional strategy addressed the issues and provided a framework for countries to develop policies to govern medicinal plants use. The strategy put forward by WHO advocates the formulation of a policy by states as the first component of developing traditional medicine, India is one of the few countries which has started to develop such policies (Prajapati, 2003). Over the past few years much research has being done and is still going on to prove scientifically the plants nutritional value and medicinal value. A good number of chemical compounds have being discovered from plants and found to have pharmacological value; this has lead to the development of over 25% of all the artificial medicines used today. Many of the traditional medicinal plants species used all over the world have being found to have great pharmacological value. Studies carried out throughout Africa confirm that indigenous plants are the main constituents of traditional medicines.

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Over 80% of the people in developing countries are using medicinal plants to treat the illnesses which affect them from day to day (Ganga, 2012). This can be attributed to poverty in these countries which has lead to inefficient health care system in hospitals and inadequate resources to access these facilities. People in these countries look for cheap and available medicines which are known traditionally to cure the illnesses. The use of herbal medicines in the western world is steadily growing with 40% of the population using plants to treat illnesses, while in Kenya 90% of the population have one time in their life used medicinal plants (Adongo et al, 2012). The use of these plants in treatment of ailments is mainly based on the type of flora in that region.

Our environment is very rich of a great range of medicinal plants and this mainly explains the reason why our grand's lived for quite some time. They could stay in the bush during war for a long period and even could use plants to treat ailments and wounds affecting soldiers in the battle ground. People all over the world should look in to their environment, especially in Africa were this information has not completely being replaced by industrial medicines, lest we forget this important aspect of treatment. Many communities in Africa still consider the use of medicinal plants as an important part of their culture, just to mention, the Maasai community in Kenya still values their culture

very much, the Kalenjn community and their medicinal fermented milk which is prepared mainly from medicinal plants such as *Senna didymobotrya* which previous studies have shown this plant to have a great potential in treatment of diseases such typhoid, diarrhea and food poisoning caused by *Salmonella typhi*, *E.coli* and *Bacillus* respectively (Ngule et al., 2013). According to Kokwaro (2009) the reason why herbal medicine still remains a matter of argument is because of some greedy practitioners who want to become wealthy by pretending to know much about the treatment of every disease that their clients complain about. This has lead to administration of wrong drugs which do not cure a patient leading to death of the individual. Proper scientific evidence needs to be provided in order to create confidence in medicinal herbs. The increase of multi-resistant strains of bacteria calls for new discoveries of new antibacterial drugs and chemical compounds that can clearly inhibit these resistant strains, this is the reason why much research should be turned to plants which have being used since ancient times to treat many diseases. (Cousins et al, 2002). The non-nutritive plant components referred to as phytochemicals are mainly attributed to the pharmacological value of medicinal plants, which can be divided in two major categories which are primary and secondary, with the primary constituting of carbohydrates, proteins and chlorophyll and the secondary consisting of tannins, alkaloids, saponins, steroids, flavonoids, terpenoids and anthroquinones (Maobe et al, 2013). The secondary metabolites help the plant survive in the environment by protecting them against predators but research has shown that these metabolites can be used to treat diseases in both animals and humans (Kokwaro, 2009). Physiological activities of phytochemicals have being found to include cancer prevention, antibacterial, antifungal, antioxidative, hormone action and enzyme stimulation.

A big percentage of plants in the savanna and semi-arid areas of East Aftica have been found to contain alkaloids which have been associated with increase in renal secretion when ingested, hence used as a diuretics and in the treatment of dropsy (Kokwaro, 2009). According to Mir (2013) the use of alkaloids, saponins and tannins as antibiotics has been scientifically justified.

Majority of the pharmacologically active chemical compounds where found mainly in ethanol extracts which is contrary to previous researches which had affirmed the traditional way of extracting these compounds using water (Iqbal, 2012).

Vernonia adoensis is used traditionally by many communities to treat various illnesses due to lack of resources to access hospitals or even preference of the use of natural medicinal plants. The roots of *Vernonia adoensis* are used traditionally mainly for the treatment of sexually transmitted diseases such as gonorrhea by the residents of Rift valley province and Western part of Kenya (Kokwaro, 2009). The plant leaves are used in the treatment of malaria. The decoction of the roots mixed with the back of other trees is used in the treatment of heart and kidney problems. According to the study carried out by Stangeland et al (2010) the plant had a very high anti-plasmodial activity and the leaves are used to treat tuberculosis. Much research has not being done to test the antibacterial activity of this plant. This study was carried out to investigate the susceptibility of the methanol-water extract of the plant against various microbes.

Material and Methods

Sample collection and preparation

The herb *Vernonia adoensis* was randomly collected in the natural forest around Baraton University 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 roots of the *Vernonia adoensis* 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 0.05 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% sulfuric 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 37^0C 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 dimethylsulfoxide (DMSO). A serial double dilution was prepared for each extract to obtain 500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml, 31.25 mg/ml, 15.63 mg/ml, 7.81 mg/ml, 3.91 mg/ml and 1.95 mg/ml respectively. An antibiotic control was made by dissolving 1µg of augmentin in 1 ml of sterile distilled water. DMSO served as a negative control.

Determination of bioactivity of the Extract

Brain heart infusion 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 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 Augmentin and DMSO control respectively. The wells were labeled on the underside of the plate. The plates were incubated at 37^0C for between 24 to 48 hours and the zones of inhibition were measured in millimeters with the aid of a ruler.

Results and Discussion

The results in table 1 have shown that the *V. adoensis* root extract controlled the growth of only *B. cereus, S. pyogenes* and *Proteus vulgaris* at zones of inhibition of 10.50±0.563, 15.67±0.422 and 11.00±0.577 respectively. All the organisms were susceptible to the antibiotic augmentin (Table 1) and unsusceptible to DMSO negative control. Analysis of variance (ANOVA) performed on the data revealed highly significant difference among the zones of inhibition at $P<0.001$. S. typhi, Klebsiella sp., E. coli and E. aerogenes were resistant to the V. adoensis extract since no zones of inhibition was observed after treatment with the extract.

The minimum inhibitory concentration (MIC) for *V. adoensis* root extract against B. cereus was 31.25 mg/ml with zone of inhibition of 10.167±0.187. The MIC for S. pyogenes was 250 mg/ml with zone of inhibition measured to be 10.00±0.089 and that of P. vulgaris at 7.67±0.145.

A Tukey's multiple comparison test has shown that there is significant difference between treatment with V. *adoensis* root extract and the control augmentin. This means that augmentin significantly controlled the bacterial growth as compared to the extract ($p<0.001$). However it was observed that V. aldoensis root extract significantly controlled Bacillus cereus growth as compared to S. pyogenes ($p<0.001$), but was not significantly different from the zone of inhibition for P. vulgaris ($p>0.05$).

Conclusion

The result obtained from the bioassay has shown that it is possible to control the spread of pathogenic microorganisms using methanol-water extract of *V. aldoensis* roots. This study is in conformity with results from other species of Vernonia against some microorganisms (Kisangau et. al., 2007). Further studies have indicated that Vernonia adoensis leaves can be boiled and the decoction is drunk to cure tuberculosis (Chitemerere and Mukanganyama, 2011). Uzoigwe and Agwa (2011) reported that Vernonia amygdalina showed significant activity *in vitro* against Klebsiella, but did not show activity against E. coli and Staphylococcus. This report is similar to the present study since V. adoensis did not show inhibition against E. coli, but different because it did not show inhibition against Klebsiella. Further study needs to be done to identify the specific compounds which are acting against the microbes.

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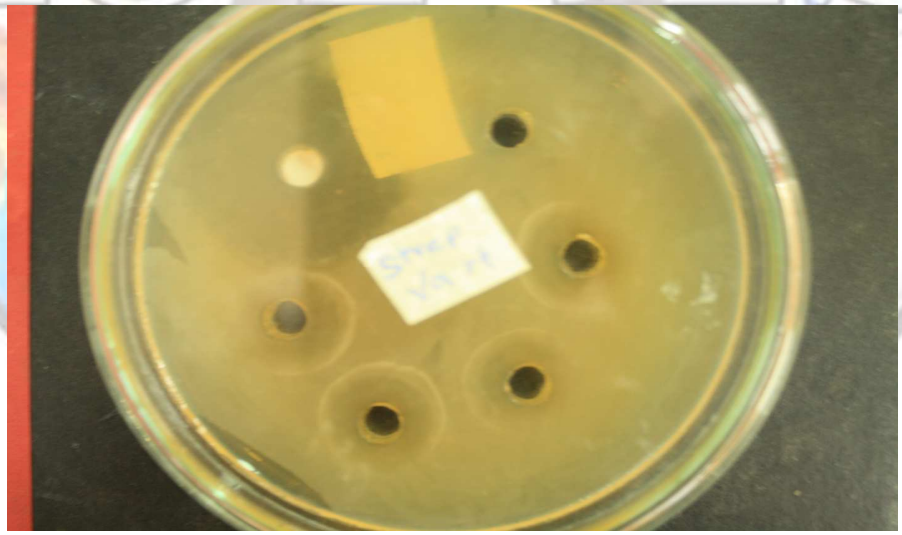

***Vernonia adoensis* roots against Streptococcus**

Table 1: Zones of Inhibition (mm ± S.E.) of 500 mg/ml of *Vernonia adoensis* root methanol extract against selected pathogenic organisms

TREATMENT ORGANISMS	VERNONIA EXTRACT	AUGMENTIN	DMSO CONTROL
Salmonella typhi	0.00±0.000	30.33±0.516	0.00±0.00
Klebsiella sp.	0.00±0.000	28.83±0.542	0.00±0.00
Bacillus cereus	10.50±0.563	28.50±0.224	0.00±0.00
Streptococcus pyogenes	15.67±0.422	29.83±0.307	0.00±0.00
Escherichia coli	0.00±0.000	38.33±0.422	0.00±0.00
Proteus vulgaris	11.00±0.577	30.33±0.422	0.00±0.00
Enterobacter aerogenes	0.00±0.000	31.00±0.447	0.00±0.00

Table 2: Tukey multiple comparison of the zones of inhibition of bacterial isolates treated with *Vernonia adoensis* extract and augmentin antibiotic control

Pairwise Comparison	P value	Significance
Salmonella typhi vs Klebsiella sp.	1.000	NS
Salmonella typhi vs Bacillus cereus	0.000	S
Salmonella typhi vs Streptococcus pyogenes	0.000	S
Salmonella typhi vs Escherichia coli	1.000	NS
Salmonella typhi vs Proteus vulgaris	0.000	S
Salmonella typhi vs Enterobacter aerogenes	1.000	NS
Klebsiella sp. vs Bacillus cereus	0.000	S
Klebsiella sp vs Streptococcus pyogenes	0.000	S
Klebsiella sp vs Escherichia coli	1.000	NS
Klebsiella sp. vs Proteus vulgaris	0.000	S
Klebsiella sp vs Enterobacter aerogenes	1.000	NS
Bacillus cereus vs Streptococcus pyogenes	0.000	S
Bacillus cereus vs Escherichia coli	0.000	S
Bacillus cereus vs Proteus vulgaris	0.999	NS
Bacillus cereus vs Enterobacter aerogenes	1.000	NS
Streptococcus pyogenes vs Escherichia coli	0.000	S
Streptococcus pyogenes vs Proteus vulgaris	0.000	S
Streptococcus pyogenes vs E. aerogenes	0.000	S
Escherichia coli vs Proteus vulgaris	0.000	S
Escherichia coli vs Enterobacter aerogenes	1.000	NS
Proteus vulgaris vs Enterobacter aerogenes	0.000	S
Salmonella typhi vs Augmentin	0.000	S
Klebsiella sp. vs Augmentin	0.000	S
Bacillus cereus vs Augmentin	0.000	S
Streptococcus vs Augmentin	0.000	S
Escherichia coli vs augmentin	0.000	S
Proteus vulgaris vs Augmentin	0.000	S
Enterobacter aerogenes vs Augmentin	0.000	S

Key: S = Significant; NS = Not Significant