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Research Article

IN VITRO CONTROL OF SELECTED PATHOGENIC MICROORGANISMS BY *VERNONIA ADOENSIS* LEAVES

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Abstract: The main aim of conducting this study was to evaluate antibacterial activity of methanol–water extract of *Vernonia adoensis* leaves against *Salmonella typi, Klebsiella* sp, *Bacillus cereus, Streptococcus pyogenes, E. coli, Proteus vulgaris and Enterobacter aerogenes*. The leaves powders 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 adoensis* leaves methanol water extract was found to control the growth of *B. cereus, Klebsiella sp., Streptococcus pyogenes* and *Escherichia coli* at zones of inhibition of 20.17 ± 0.307, 17.33 ± 0.211,11.67 ± 0.494 and 12.17 ± 0.477 respectively. From the study methanol-water extract can be used to control *B. creus, Klebsiella sp., Streptococcus pyogenes* and *Proteus vulgaris*. More research needs to be done to identify the specific bioactive compounds

Keywords: *Vernonia adoensis, Antibacterial activity, Extract, Pathogenic micro-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 have 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 led 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.

Over 80% of the people in developing countries are using medicinal plants to treat the illnesses which affect them (Ganga, 2012). This can be attributed to poverty in these countries which has led 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

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such typhoid, diarrhea and food poisoning caused by *Salmonella typi, E.coli and Bacillus cereus 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 Africa where Kenya is located 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 leaves 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 leaves 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°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°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° 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°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

Table.1: Zones of inhibition (mm ± S.E.) of Vernonia adoensis leaves extract against selected pathogenic microorganisms and augmentin control.

Treatment / Organisms	Vernonia Leaves Extract	Augmentin	DMSO Control
Salmonella typhi	0.00±0.000	32.83±0.307	0.00±0.000
Klebsiella sp.	17.33±0.211	28.83±0.307	0.00±0.000
Bacillus cereus	20.17±0.307	28.67±0.333	0.00±0.000
Streptococcus pyogenes	11.67±0.494	28.43±0.297	0.00±0.000
Escherichia coli	12.17±0.477	37.00±0.365	0.00±0.000
Proteus vulgaris	0.00±0.000	30.50±0.428	0.00±0.000
Enterobacter aerogenes	0.00±0.000	31.17±0.307	0.00±0.000

Key: SE=standard error; DMSO=Dimethyl sulfoxide

Vernonia adoensis against Bacillus cereus

A one way analysis of variance (ANOVA) showed that there was significant difference in the zones of inhibition obtained for the extract and the antibiotic control ($p<0.001$). There was no growth observed for the DMSO controls on any of the plates for any of the organisms.

The zones of inhibition of *B. cereus* give the best result, follow by that of *Klebsiella sp.*, then *Escherichia coli* and then *S. pyogenes* (table 1). Augmentin however significantly controlled the growth of the organism as compared to the extract. Zones of inhibition greater than 8mm for the extract is considered active, therefore, the extract is considered active for all the organisms that it showed growth against, but remain inactive for *Salmonella typhi, Proteus vulgaris* and *Enterobacteraerogenes* in this study.

Table.2: Tukey's pair wise comparison of the zones of inhibition for *Vernonia adoensis* leaves extract against selected microorganisms and augmentin control.

Pairwise Comparison	P value	Significance
Salmonella typhivs Klebsiella sp.	0.000	S
Salmonella typhivs Bacillus cereus	0.000	S
Salmonella typhivs Streptococcus pyogenes	0.000	S
Salmonella typhivs Escherichia coli	0.000	S
Salmonella typhivs Proteus vulgaris	1.000	NS
Salmonella typhivsEnterobacteraerogenes	1.000	NS
Klebsiella sp. vs Bacillus cereus	0.000	S
Klebsiellaspvs Streptococcus pyogenes	0.000	S
Klebsiellaspvs Escherichia coli	0.000	S
Klebsiella sp. vs Proteus vulgaris	0.000	S
KlebsiellaspvsEnterobacteraerogenes	0.000	S
Bacillus cereus vs Streptococcus pyogenes	0.000	S
Bacillus cereus vs Escherichia coli	0.000	S
Bacillus cereus vs Proteus vulgaris	0.000	S
Bacillus cereus vs Enterobacteraerogenes	0.000	S
Streptococcus pyogenesvs Escherichia coli	0.997	NS
Streptococcus pyogenesvs Proteus vulgaris	0.000	S
Streptococcus pyogenesvs E. aerogenes	0.000	S
Escherichia coli vs Proteus vulgaris	0.000	S
Escherichia coli vs Enterobacteraerogenes	0.000	S
Proteus vulgaris vs Enterobacteraerogenes	1.000	NS
Salmonella typhivs 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 vsaugmentin	0.000	S
Proteus vulgaris vs Augmentin	0.000	S
Enterobacteraerogenesvs Augmentin	0.000	S

Key: S = Significant; NS = Not Significant

A pair wise comparison showed significant differences between the extract and various organisms and no significance were observed only with *Salmonella-Proteus* and *Salmonella-Enterobacter* pairs because the three organisms were not susceptible to the extract. *S. pyogenes* and *E. coli* significantly controlled the growth similarly with no significant difference in their inhibition zones at $p>0.05$. *Bacillus cereus* however was significantly reduced by the *Vernonia* leaves abstract as compared to all the other organisms ($p<0.05$).

The results have shown that a formulation produced from *V. adoensis* leaves can significantly control infections caused by S.pyogenes, including endocarditis, pharyngitis and several other respiratory conditions.

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 *Vernonia adoensis* leaves. 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*. A study on *Vernonia adoensis* roots have shown that the roots of plant inhibits the growth of Bacillus cereus, Streptococcus pyogenes and proteus vulgaris but did not show inhibition against *salmonella typi, klebsiella sp, Escherichia coli* and *Enterobacter aerogenes.* This contrally to the expectations in which the leaves of the same species have being found to inhibit the growth of *Echerichia coli* and *Klebsiella sp* but did not inhibit the growth of Proteus vulgaris (T. Anthoney Swamy *et al.,* 2013). This difference in antibacterial activity can be attributed to the difference in the phytochemical composition of the roots and leaves of the plant. The study done by T. Anthoney Swamy *et al.,* (2013) shows that alkaloids and terpenoids were found on the leaves but absent in the roots. From these two studies it is clear that different phytochemicals have different pharmacological values and therefore research needs to be done to make these differences in activity clear and applied to treat diseases caused by the microbes. *Vernonia adoensis* leaves have shown great pharmacological value in treating against food poisoning, stomach problems and sore throat infections. Further study needs to be done to identify the specific compounds which are acting against the microbes.

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