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IN VITRO ANTIBACTERIAL ACTIVITY OF METHANOLIC – AQUA EXTRACT OF *PLECTRANTHUS ARGENTATUS* LEAVES

Anthoney Swamy T[1*], Jackie Obey K[2] and Ngule Chrispus Mutuku[3]

[1* & 3]Department of Chemistry, University of Eastern Africa Baraton, P.O. Box 2500, Eldoret -30100, Kenya.

[2] Department of Medical Lab Science, University of Eastern Africa Baraton, P.O. Box 2500, Eldoret -30100, Kenya.

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***Correspondence for Author:**
Dr. Anthoney Swamy T,
Department of Chemistry,
University of Eastern Africa
Baraton, P.O. Box 2500,
Eldoret -30100, Kenya.
drtanthony2011@yahoo.com

ABSTRACT

The study was conducted to analyze the antibacterial activity of *Plectranthus argentatus* plant leaves. The plant sample was extracted using methanol and water in the ratio 9:1. From the study the plant *Plectranthus argentatus* was found to inhibit the growth of *Escherichia coli* with a zone of inhibition of 11.67 ± 0.882, *Serratia liquefaciens* zone of inhibition of 12.67 ± 0.667, *Bacillus cereus* 28.00 ± 1.154 and *Proteus vulgaris* 11.00 ± 0.577. The bacteria which had a zone of inhibition of less than 8 mm were termed to be unsusceptible. The data collected and documented in this paper is a scientific justification that the plant *Plectranthus argentatus* can be used to treat against various diseases caused *by E.coli, S. liquefaciens, Bacillus cereus and Proteus vulgaris.* However, more scientific data needs to be provided to indicate the mode action of the plant active compounds against the pathogens.

Keywords: *Plectranthus argentatus, Antibacterial, Medicinal herbs, Leaves.*

INTRODUCTION

Study on medicinal plants is becoming an important area of study. Micro-organisms are becoming resistant to drugs used to kill them, hence the need for alternative drugs to treat them. Many scientists have turned to plants to get these compounds. The use of medicinal plants to treat various types of diseases was very important in ancient days since there were no commercial medicines by then. The introduction of Synthetic drugs has however led many

people to turn from plants to use these synthesized products. However, the trend is changing with many turning to plants for treatment.

Pharmacological studies have reported appealing results showing the importance of using plant extract to treat diseases. Pharmacological studies report on endangered species *Pontella fulgens* have indicated that the plant can be used as an antitumor, anti-inflammatory, anti-hyperlipidemic, and anti-hyperglycemic and hypoglycemic [1]. The antibacterial activity of plants such as *Senna didymobotrya* has been associated with the presence of certain phytochemicals such as tannins and alkaloids [2&3]. The plant showed clear zones of inhibition against *B.subtillis, E.coli, P.aeruginosa and C.albicans.* The plant also showed a great potential in the treatment against animal wounds [4]. All these activity against microbes have been closely attributed to the presence of phytoconstituents.

All chemicals found in plants are potential drugs. Certain tree barks produce a chemical that discourage caterpillars from feeding on them a good example being the Indian neem tree which keeps off desert locusts. The twigs are chewed by people in Serengeti national park in east Africa to prevent tooth decay. Plants produce more than 10,000 different compounds to prevent themselves against animal who feed on them. Almost half of all prescribed drugs contain chemicals produced by plants, fungi and bacteria or contain synthesized compounds in the laboratory that have been modeled after plants originating compounds [5].

The use of medicinal plants to treat diseases is as old as man. Medicinal plants have been used since ancient times to treat many illnesses [6]. Research has shown that the concentration of these compounds in plants is directly related to their capability to treat certain illness. Many of these non-nutritive secondary metabolites are found in plants which are even used for food. Over 80% of the plants in Nigeria used for treatment of malaria and other sicknesses are also used as food [7]; 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 [8]. Over the past few years much research has been done and is still going on to prove scientifically the plants nutritional value and medicinal value. A good number of

chemical compounds have been 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 throughout Africa confirm that indigenous plants are the main constituents of traditional African medicines.

Over 80% of the people in developing countries use medicinal plants to treat the illnesses which affect them from time to time [9]. 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 has one time in their life used medicinal plants [10]. 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 with 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 some time and even could use plants to treat ailments and wounds affecting soldiers in the battle ground. People all over the world should look around them especially in Africa were this information has not completely being replaced by industrial medicines, lest we forget this important aspect of treatment. In many communities in Africa they still consider the use of medicinal plants as an important part of their culture, just to mention, the Maasai community in Kenya still value their culture very much, the Kalenjn community and their medicinal fermented milk which is prepared mainly from medicinal plants such as *Senna didymobotrya stem* which our previous studies have shown this plant to have a great potential in treatment of diseases such as typhoid, diarrhea and food poisoning caused by *Salmonella typhi*, *E.coli and Bacillus cereus* respectively [2]. 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 [11]. This has led to administration of wrong drugs which do not cure the patient leading to worsening of the condition or even 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 antibacterial classes and

chemical compounds that can clearly inhibit these resistant strains, this is the reason why much research should be turned to plants which have been used since ancient times to treat many diseases [7].

The non-nutritive plant components are referred to as phytochemicals, which can be divided in two major categories 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 [12]. 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 [11]. The antibacterial activity of plants have been closely associated with the presence of these important compounds in the plant. *Vernonia adoensis* leaves against *B.cereus, Klebsiella sp., Streptococcus pyogenes* and *Proteus vulgaris* is closely associated to the presence of phytochemicals in the plant leaves extract [13]. Physiological activities of phytochemicals have been found to include cancer prevention, antibacterial, antifungal, anti-oxidative, hormone action and enzyme stimulation.

Natural bioactive compounds have been investigated in plants and their pharmacological effects analyzed. Secondary metabolites functions on growth, photosynthesis and other important plant activities have not been discovered but their medicinal values have been identified in most of them [14]. Phytochemicals have been used to a greater extend in Asia for various purposes such as treatment of diseases [15].

The lack of scientific knowledge on the phytochemical constituents, antibacterial, antioxidants and toxicological properties limits the use of traditional herbal medicine [3]. Phytochemicals can really improve the activity of the currently used drugs by acting as efflux of existing pump inhibitors. Many drug resistant microbes are emerging from time to time and causing the need to such for new antibiotics to kill and inhibit their growth. Phytochemicals have been associated with reduction of drug resistant forms of bacteria [16].

A big percentage of plants in the savanna and semi-arid areas of east Africa contains alkaloids which have been associated with increase in renal secretion when ingested, hence used as a diuretics and in the treatment of dropsy [11]. The use of alkaloids, saponins and tannins as antibiotics has been scientifically justified [6].

Majority of the pharmacologically active chemical compounds were found mainly in ethanol extracts which is contrary to previous researches which had affirmed the traditional way of extracting these compounds using water [17].

Plectranthus argentatus leaves are used by the Kisii community in Kenya to treat against stomach pain and inflammation. This study was carried out to investigate the antibacterial activity of the plant.

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 collected and 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 powdered leaves of the *Plectranthus argentatus* was 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, and kept for 24 hours on a shaker for effective extraction of the plant components. The extract was filtered using Butchner funnel; Whatman no.1 filter paper and 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 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, a procedure similar to that used by *Biruhalem* [18] and Donay *et. al.*,[19]. 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% 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 37^0C 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). 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 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 Augmentin and DMSO control respectively. Three plates were made for each bacterial organism and extract giving a triplicate reading for each microorganism and extract. 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 DISCUSION

Table 1: Zones of Inhibition (mean ± S.E.) of 500 mg/ml of *Plectranthus argentatus* Extract Against Selected Microorganisms.

Microorganisms	Mean ± S.E.	Penicillin	DMSO control
Escherichia coli	11.67±0.882	40.00±0.000	0.00±0.000
Salmonella sp.	0.00±0.000	31.00±0.000	0.00±0.000
S. liquefaciens	12.67±0.667	36.00±0.000	0.00±0.000
Enterobacter sp.	5.67±2.962	40.00±0.000	0.00±0.000
Bacillus cereus	28.00±1.154	45.00±0.000	0.00±0.000
Proteus vulgaris	11.00±0.577	46.00±0.000	0.00±0.000

The average mean zone of inhibition (±S.E.) was calculated for each of the microbial organism. The zones of inhibition of the microorganisms were also analysed by analysis of variance (ANOVA) and it was shown that there were significant differences in the zones of inhibition among the microbial organisms ($p<0.05$). The biggest zone of inhibition was against *B. cereus* (28.00±1.154) followed by *S. liquefaciens* (12.67±0.667)), *Proteus vulgaris* and *Escherichia coli* (11.67±0.882). Zones of inhibition recorded for the other organisms were not considered significant since they measured below 8mm. Several species of *Plectranthus* have exhibited antimicrobial activity. Essentials oils extracted from *P. ornatus, P. amboinicus* and *P. barbatus* were 512, 256 and 512 µg/ml respectively for E. coli, 128, 64 and 256ug/ml respectively for P. vulgaris and 512, 512 and 64 ug/ml respectively for B. cereus [20]. In another study, methanol extract of *P. amboinicus* showed zones of inhibition of 13±0.21, 14±0.15 and 11±0.24 against *S. aureus, B. cereus* and *P. aeruginosa* respectively [21]. Abietanes extracted from the acetone extracts of *P. grandidentatus* and *P. hereroensis* have shown activity against methicillin resistant *Streptococcus aureus* with MIC values ranging between 0.98-15.63µg/ml and vancomycin resistant *Enterococcus faecalis* with MIC values of 15.63 and 31.25 µg/ml [22]. Zones of inhibition ranging from 12 to 20mm is considered strongly active, 8 to 10mm moderately active and 7mm and below low active, when crude extractions are examined for antibacterial activity.The compound 7a-acetoxy-6b-hydroxyroyleanone isolated from *Plectranthus sp.* has shown a MIC value of 31.25 against *S. aureus* [23]. *Plectranthus cylindraceus* has aslo showed antifungal activity with MIC values ranging from 7.8 to 62.5 ug/ml against *Microsporum canis, Microsporum gypseum and Trichophyton rubrum, Alternaria alternata, Bipolaris sp., Curvularia lunata* and *Fusarium oxysporum* [24]. Several other plants in Puerto Rico have also been studied for antimicrobial activity including *Punica granatum, Citrus aurantium, Tamarindus indica* among other plants [25].

Table 2: Tukey's Honestly Significant Difference Among Microorganisms. (Using 500mg/ml of *Plectranthus argentatus* Extract)

Comparison	P Value	Significance
E. coli vs Salmonella sp	0.001	S
E. coli vs. *S. liquefaciens*	0.995	NS
E. coli vs E. aerogenes	0.084	NS
E. coli vs. Bacillus cereus	0.000	S
E. coli vs Proteus vulgaris	0.999	NS
Salmonella sp. vs *S. liquefaciens*	0.000	S

Salmonella sp. vs. E. aerogenes	0.111	NS
Salmonella sp. vs B. cereus	0.000	S
Salmonella sp. vs P. vulgaris	0.001	S
S. liquefaciens vs. E. aerogenes	0.036	S
S. liquefaciens vs. B. cereus	0.000	S
S. liquefaciens vs. P. vulgaris	0.953	NS
E. aerogenes vs. B. cereus	0.000	S
E. aerogenes vs. P. vulgaris	0.145	NS
B. cereus vs. P. vulgaris	0.000	S

The study shows that the plant *Plectranthus argentatus* can inhibit the growth of four microorganisms out of the six it was tested against. The plant show clear zones of inhibition against *Escherichia coli* with a zone of inhibition of 11.67 ± 0.882, *S. liquefaciens* 12.667 ± 0.667, *Bacillus cereus* 28.00 ± 1.154 and *Proteus vulgaris* 11.00 ± 0.577. Comparing for the mean zones of inhibition of 500 mg/ml of *Plectranthus argentatus* showed that there was significant difference in the zones of inhibition among the organisms $p<0.001$. On further comparison using the Tukey's pairwise comparison showed that zones of inhibition for *E. coli* were significantly higher than those of Salmonella sp. and *P. vulgaris* ($p<0.05$). Zones of inhibition of *Salmonella* were significantly lower than all the organisms ($p<0.05$) except for *E. aerogenes*. Significant difference were also observed between *B. cereus* and all the organisms ($p<0.05$). The antibacterial activity of the plant can be attributed to the presence of the phytochemicals found in the plant [26], from the study the plant was found to contain tannins, flavonoids, cardiac glycosides, alkaloids, saponins, phenols, and steroidal rings, but terpenoids, steroids and steroidal nucleus were found to be absent. The phytochemicals found in the plant have been investigated and found to have antibacterial activity therefore justifying traditional plant use to treat against various diseases caused by bacteria. The plant has shown great pharmacological value.

CONCLUSION

The data provided in this study is scientific justification that the plant *Plectranthus argentatus* can be used to treat against diseases such as abdominal cramps and diarrhea caused by *Bacillus cereus* due to its ability to cause food poisoning, treat against *Escherichia coli* which causes serious and even life threatening effects such as hemolytic-uremic syndrome (HUS), diarrhea and neonatal meningitis. It can also be used to treat against blood stream infections and urinary tract infections caused by *Serratia liquefaciens* , opportunist pathogen *Proteus vulgaris* which causes wound infections. From the study the plant *Plectranthus argentatus* has shown to have great medicinal value and therefore justifying its

traditional use to treat against various diseases. More research needs to be done to identify the mode of action of the active compounds in the plant.

Plectranthus argentatus plant

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