INTERNATIONAL JOURNAL OF BIOASSAYS
ISSN: 2278-778X
CODEN: IJBNHY
ORIGINAL RESEARCH ARTICLE
OPEN ACCESS

IN VITRO ANTIBACTERIAL ACTIVITY OF THE AQUA EXTRACT OF *PHYTOLACCA DODECANDRA* ROOTS AGAINST LABORATORY STRAINS OF SELECTED HUMAN PATHOGENIC ORGANISMS

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Received for publication: February 26, 2015; **Accepted:** April 22, 2015

Abstract: The aim of this study is to test the antibacterial activity of the aqua extract of *Phytolacca dodecandra* roots against *Bacillus cereus, Staphylococcus epidermidis, Streptococcus pyogenes, Escherichia coli, Serratia marcescens, Serratia liquefaciens, Salmonella typhi* and *Enterobacter aerogenes*. The agar well diffusion method was used to determine the zones of inhibition by the extract, penicillin antibiotic (positive control) and sterile distilled water (negative control) against the organisms. The extract showed the highest activity against *Enterobacter aerogenes* (16.333±0.882 mm), followed by *Bacillus cereus* (14.33±0.333 mm), *Escherichia coli* (14.00±0.000 mm), *Streptococcus pyogenes* (13.00±0.000 mm). The zone of inhibition against *Serratia marcescens* was 7.33±0.333 mm, considered inactive because it is less than 8 mm. All the other organisms were not inhibited by the extract. The Tukey's significant difference test showed significant differences with most of the pair-wise comparisons while a few zones were not significantly different from the other. The results from this study have shown that the aqua extract of *P. dodecandra* roots can inhibit both gram negative and gram positive bacteria *in vitro*. The pharmacologic mechanisms of action of the active ingredients must be studied and the active components can be considered for possible incorporation into conventional antibacterial drug production.

Keywords: Antibacterial, pathogenic organisms, *Phytolacca, dodecandra,* roots, aqua

INTRODUCTION

In continuation with our interest in the study on medicinal plants [1-12], we take up on antibacterial activity of aqua extract of *Phytolacca dodecandra* roots. 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, hepato protective activity, haemolytic activity, larvicidal activity, anthelmintic activity, pain relief activity, central nervous system activity, sexual impotence and erectile dysfunction and hypolipidemic activity [13,14,15,16,17,18,19 & 20]. 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 [21, 22 & 23]. The nature is a paradise which offers medicinal principles to humanity through plants [24].

Traditional medicine includes herbal medicines composed of herbs, herbal materials and herbal products [25]. Humans and animals have been using natural medicinal plant's part such as roots, leaves, barks, seeds and flowers [26]. Most developing countries, especially those in Asia, Africa, Latin America and the Middle East people (about 70%-95% population) are using TM as medicinal treatment for various diseases [27].

The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant which used a sources of medicines throughout history and continued to serve as the basis for many pharmaceuticals used today [28].

The *P. dodecandra* roots are used in small quantities as a purgative, taenifuge and cathartic. The roots are dug and pounded; a little is mixed with water and the extract drunk in reasonable quantities (two table-spoonfuls) daily. This is for the treatment of roundworm and tapeworm. If taken excessively it can be poisonous and may cause death. A decoction of the roots is also drunk to cause vomiting, for the treatment of enlarged glands. The roots are used as cure for venereal diseases, particularly syphilis. In this case the roots are boiled and the decoction drunk. One pint of the infusion is normally enough. In some people this causes vomiting, but if this should cause diarrhea then it is sign of an overdose and the patient should immediately be given fresh milk, leaf infusion give to cows to remove retained placenta. Leaf infusion also used for washing livestock to kill ticks. We must

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however, bear in mind that the plant, especially the leaves and roots, is very toxic and must be used in very small amounts [29].

Berries from *P. dodecandra* offer a readily available molluscicide to control schistosomiasis. Parts of the *endod* plant have been used as a detergent and as traditional medicine for centuries in Ethiopia. An interview survey was performed in the highlands of Ethiopia to provide information on the distribution of the plant, people's traditional use of it, their perception of the plant, and the potential for increased production and use of *endod* as soap for indirect control of schistosomiasis. People of all ages report that they are familiar with the plant and its detergent and medicinal uses. The plant is largely disappearing from unprotected areas due to land clearing. Younger people appear to use *endod* as soap whenever it is available. Older women prefer commercial soap and consider *endod* to be associated with poor people. Common medicinal uses include treatment of skin itching (ringworm), abortion, gonorrhea, leeches, intestinal worms, anthrax and rabies [30].

Antirabies activity of hydro-ethanolic extract of roots and leaves of *Phytolacca dodecandra* (L' Herit), one of the widely used plants for traditional treatment of rabies in humans and animals in Ethiopia, by using mice model [31].

Various traditional antirabies plants were reported in different indigenous people and parts of Ethiopia which were used for the treatment of rabies in humans and animals. Among the reported plants, *P. dodecandra* is widely used for the traditional treatment of rabies in human and animals in Ethiopia. However, a little work was done on evaluating the efficacy of these and other unidentified ethno-medicinal and ethona-veterinary plants used to treat rabies cases [31].

The aim of the present study is to analyse the antimicrobial activity of the aqua extract of *Phytolacca dodecandra* roots against the laboratory strains of selected human pathogenic organisms.

MATERIAL AND METHODS

Sample collection and Extraction procedure

The *Phytolacca dodecandra* root was collected around Baraton University. The samples were identified by a taxonomist at the University of Eastern Africa, Baraton. The fresh root of the *P. dodecandra* was air-dried for three weeks; the dried root was ground into powder. Forty grams (40g) of the powdered bark were mixed with 400 ml of water. The mixture was kept for 24 hours on a shaker for effective extraction of the plant components. The extract was filtered and the water was evaporated to dryness at a temperature of 40°C using rotary vacuum evaporator. The extract was brought to dryness using vacuum and pressure pump. The yield was kept at 4°C prior to use.

Test for microorganism

The microorganisms used in this investigation were *Bacillus cereus, Staphylococcus epidermidis, Streptococcus pyogenes, Escherichia coli, Serratia marcescens, Serratia liquefaciens, Salmonella typhi* and *Enterobacter aerogenes.*

Preparation of the Extract Concentrations and Antibiotic

A stock solution for the extract was prepared by dissolving 500 mg in 1 ml of sterile distilled water. An antibiotic control (positive control) was prepared by dissolving 1mg of penicillin in 1 ml of sterile distilled water. Sterile distilled water served as a negative control.

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). Sulphuric acid (1%) was prepared in a 100-ml volumetric flask. To prepare the 0.5 McFarland Standard, 0.5 ml of the 1% $BaCl_2$ solution was mixed with 99.5 ml of H_2SO_4 solution. Measure the turbidity of the 0.5 McFarland Standards with the aid of a spectrophotometer at a wavelength of 625 nm to read an optical density of between 0.08 to 1.0. At this absorbance, the McFarland standard represents a bacterial cell density of approximately 1.5 x 10^8 CFU/ml (1.0 x 10^8 – 2.0 x 10^8 CFU/ml). It was then transferred to a screw-capped bottle and sealed with parafilm to prevent evaporation due to exposure to air. The bacterial suspensions were then tested against the McFarland standards until they reached the absorbance of the McFarland standard and then they were ready for use.

Screening for the antibacterial potential of the plant extract

The agar well diffusion procedure was used in the experiment [32 & 33]. The microorganisms used for this study were laboratory strains of *Bacillus cereus, Escherichia coli, Salmonella typhi, Streptococcus pyogenes, Serratia marcescens, Serratia liquefaciens, Enterobacter aerogenes and Staphylococcus epidermidis.* A single colony for each of the organisms was picked from Mueller Hinton agar plate and dissolved in 5 ml of Mueller Hinton broth. The broth was incubated overnight at 37°C. Five millilitres (5 ml) of plain Mueller Hinton broth was incubated alongside the organisms to ensure that the medium was not contaminated. The

UV spectrophotometer was set to 625 nm wavelength and each of the microbial cultures was pipetted into cuvette to measure the absorbance. A cuvette of plain Mueller Hinton broth was used as a blank at 0.000 absorbance. The absorbance of the microorganisms was measured. The bacterial organisms exceeding 0.1 absorbance were adjusted by adding bacterial suspension until the absorbance fell between 0.08 to 0.10, matching the 0.5 McFarland Standard. The organisms falling below 0.08 absorbance were also adjusted until the McFarland standard absorbance was achieved. All the organisms, therefore, reached a cell density of 1 x 10^8 cfu/ml [34]. One hundred (100) µl of each of the organisms were then inoculated onto agar plates for the bioassay [35]. Three 6 mm wells were made into each agar plate using a sterile metal cork borer. One hundred micro litres (100 µl) of the standard drug penicillin was placed in one well, the extract in another well and sterile distilled water was placed in the third well on each plate. The experiment was run in triplicate for each extract and each organism tested. The plates were incubated for 24 to 48 hours and the zones of inhibition were measured in millimetres with the aid of a meter rule.

Determination of relative percentage inhibition

The relative percentage inhibition of the test extract with respect to positive control was calculated by using the following formula [36 & 37].

$$\text{The relative percentage inhibition of the test extract} = \frac{100\ x\ (X-Y)}{(Z-Y)}$$

Where,

X: total area of inhibition of the test extract
Y: total area of inhibition of the solvent
Z: total area of inhibition of the standard drug

The total area of the inhibition was calculated by using area = πr^2 ; [Where, r = radius of zone of inhibition].

Statistical Analysis

A random sampling procedure was done for the entire test and the experiment was conducted in triplicate assays on Mueller Hinton agar plates [33]. The mean values and standard error were calculated for the zones of inhibition. Analysis of variance was used to determine if there was significant difference among the average zones of inhibition of the bacterial organisms by the extract and controls. The Tukey's honestly significant difference test was used to determine pair-wise comparisons between average zones of inhibition among the bacterial organisms by SPSS version 21.0.

RESULTS AND DISCUSSION

The results from this study have shown that the aqua extract of *P. dodecandra* root can inhibit both gram negative and gram positive bacteria *in vitro*. The aqueous extract of *Phytolacca dodecandra* was active against *Enterobacter aerogenes, Bacillus cereus, Escherichia coli* and *Streptococcus pyogenes*. The zones of inhibitions were 16.333 ± 0.882 mm for *E. aerogenes*, 14.333 ± 0.333 mm for *B. cereus*, 14.000 ± 0.000 mm for *E. coli* and 13.000 ± 0.000 mm for *S. pyogenes* (Table 1). The zone of inhibitions against *S. marcescens* was 7.333 ± 0.000 mm, considered inactive because it is less than 8 mm. All the other organisms had zone of inhibitions 0.000 ± 0.000. All the zones of inhibitions of extracts were smaller in zones of inhibition than the positive control penicillin antibiotics whereas the negative control (sterile distilled water) did not show any zone of inhibition 0.000 ± 0.000. ANOVA results showed that all the organisms were significantly different from each other and their controls (P < 0.001).

Table 1: Zone of inhibition (mm ± S.E.) of aqua extract of *P. dodecandra* roots.

Microorganism	Zone of Inhibition (mm ± S.E.)	Penicillin Control (mm ± S.E.)	Negative control (mm ± S.E.)
Bacillus cereus	14.333 ± 0.333	34.333 ± 0.333	0.00 ± 0.000
Staphylococcus epidermidis	0.000 ± 0.000	40.000 ± 0.000	0.00 ± 0.000
Streptococcus pyogenes	13.000 ± 0.000	37.166 ± 0.167	0.00 ± 0.000
Escherichia coli	14.000 ± 0.000	40.000 ± 0.000	0.00 ± 0.000
Serratia marcescens	7.333 ± 0.000	36.000 ± 0.000	0.00 ± 0.000
Serratia liquefaciens	0.000 ± 0.000	38.667 ± 0.667	0.00 ± 0.000
Salmonella typhi	0.000 ± 0.000	40.000 ± 0.000	0.00 ± 0.000
Enterobacter aerogenes	16.333 ± 0.882	34.333 ± 0.333	0.00 ± 0.000

Key: S.E. = standard error;

Tukey's test has shown that the zones of inhibition of *B.cereus* was not significantly different from that of *S. pyogenes* and *E. coli* (P > 0.05) but was significantly smaller that of *E. aerogenes* (P < 0.05). The zone of inhibition for *B. cereus* is significantly bigger than those of *S. epidermidis. S. marcescens, S. liquefaciens* and *S. typhi* (Table 2). All the bacteria had zones of inhibition significantly smaller than their control (P < 0.001).

The zones of inhibition of *S. epidermidis* was significantly smaller than those of *S. pyogenes, E. coli, S. marcescens* and *E. aerogenes* and its antibiotic control (P < 0.05) but it was not significantly different from those of organism such as *S. liquefaciens* and *S. typhi* (P > 0.05).

Table 2: Tukey's honestly significant differences between microorganisms treated with the aqua extract of *P. dodecandra* roots and antibiotic control.

COMPARISON	P- value	SIGNIFICANCE
B. cereus vs. S. epidermidis	0.000	S
B. cereus vs. S. pyogenes	0.270	NS
B. cereus vs. E. coli	1.000	NS
B. cereus vs. S. marcescens	0.000	S
B. cereus vs. S. liquefaciens	0.000	S
B. cereus vs. S. typhi	0.000	S
B. cereus vs. E. aerogenes	0.010	S
B. cereus vs. B. cereus control	0.000	S
S. epidermidis vs. S. pyogenes	0.000	S
S. epidermidis vs. E. coli	0.000	S
S. epidermidis vs S. marcescens	0.000	S
S. epidermidis vs. S. liquefaciens	1.000	NS
S. epidermidis vs. S. typhi	1.000	NS
S. epidermidis vs. E. aerogenes	0.000	S
S. epidermidis vs. S. epidermidis control	0.000	S
S. pyogenes vs. E. coli	0.708	NS
S. pyogenes vs. S. marcescens	0.000	S
S. pyogenes vs. S. liquefaciens	0.000	S
S. pyogenes vs. S. typhi	0.000	S
S. pyogenes vs. E. aerogenes	0.001	S
S. pyogenes vs. S. pyogenes control	0.000	S
E. coli vs. S. marcescens	0.000	S
E. coli vs S. liquefaciens	0.000	S
E. coli vs. S. typhi	0.000	S
E. coli vs. E. aerogenes	0.000	S
E. coli vs. E. coli control	0.000	S
S. marcescens vs. S. liquefaciens	0.000	S
S. marcescens vs. S. typhi	0.000	S
S. marcescens vs. E. aerogenes	0.000	S
S. marcescens vs. S. marcescens control	0.000	S
S. liquefaciens vs. S. typhi	1.000	NS
S. liquefaciens vs. E. aerogenes	0.000	S
S. liquefaciens vs. S. liquefaciens control	0.000	S
S. typhi vs. E. aerogenes	0.000	S
S. typhi vs. S. typhi control	0.000	S
E. aerogenes vs. E. aerogenes control	0.000	S

Key: NS = Not Significant; S = Significant

The zones of inhibitions of S. pyogenes were significantly larger than those of S. marcescens, S. liquefaciens and S. typhi but significantly smaller than its antibiotic control and *E. aerogenes.* The zone of inhibition of *S. pyogenes* was not significantly different from *E. coli.*

The zones of inhibitions of *E. coli* were larger than *S. marcescens, S. liquefaciens* and *S. typhi* but significantly smaller than its control and *E. aerogenes.* The zones of inhibitions of *S. marcescens* were significantly larger than those of *S. liquefaciens* and *S. typhi* but smaller than its antibiotics control and *E. aerogenes*.

The zone of inhibition of *S. liquefaciens* was similar to that of *S. typhi* but significantly smaller than those of its control and *E. aerogenes.* The zones of inhibitions of *S. typhi* were significantly smaller than its control and *E. aerogenes.* And the zone of inhibition of *E. aerogenes* was significantly smaller than its control. There was no zone of inhibition produced by negative control (sterile distilled water) against any of the organisms.

The *Phytolacca dodecandra* leaf extracts had higher antibacterial activity as compared to antifungal activity. The methanol extract of *P. dodecandra* stem bark was the most active against *Salmonella typhi* and *P. aeruginosa.* Dichloromethane and ethyl acetate extracts of the roots of *P. dodecandra* were active against *P. aeruginosa.* The aqueous extract had very mild activity against *E. coli* while ethyl acetate also had mild activity against *Salmonella typhi* [38].

The methanolic extract of *P. dodecandra* root had strong activity against *S. pyogens* and *P. aeuruginosa* comparable to the root of *B. antidysenterica* [39]. Similarly, its petroleum ether extract was indicated to be active against gram-positive bacteria with inhibition diameter ranging from 12 to 20 mm [40].

The MIC of *P. dodecandra* against the dermatophytes tested ranged from 19.5 mg/l to 156.0 mg/l. The minimum inhibitory concentration for 50% (MIC50) of the dermatophyte strains was 62.5 mg/l. The extract showed fungicidal activity against the dermatophytes that ranged from 19.5 to 312.5 mg/l [41].

The aqueous and butan-1-ol extracts of *P. dodecandra* were evaluated for their effects on the isolates of HCF and for the treatment of cases of EL. The antifungal effect of butan-1-ol extract was observed to be much greater than that of the aqueous extract EL [42]. The minimum inhibitory concentration of butan-1-ol extract ranged from (0.039%–0.078%); whereas that of the aqueous extract was in the range of (0.625%–1.250%). Similar result was observed for the aqueous extract in which the minimum inhibitory concentration of *P. dodecandra* against the yeast forms of different *Candida* species were higher than 0.5% [41] and another study showed that the minimum inhibitory concentration of the aqueous extract of *P. dodecandra* was 1% [43]. The minimum inhibitory concentration for novel pharmacological compounds should be less than 0.1% [44]. Since minimum inhibitory concentration of the aqueous extract of *P. dodecandra* against HCF was greater than the recommended level (0.1%), this extract could be considered inactive. On the other hand, the minimum inhibitory concentration of butan-1-ol extract of *P. dodecandra* was found to be below 0.1%, hence, the extract could be considered active.

The MFCs of aqueous and butan-1-ol extract of *P. dodecandra* ranged from (1.250%–2.500%) and (0.078%–0.156%), respectively. Similar results were reported against different dermatophyte strains of human pathogen [41]. The MFC and MIC of butan-1-ol

extract were found to be higher than the standard indicating that it is less potent as compared to the standard.

According to Arif [45], the phytochemical analysis of *P. dodecandra* showed the presence of saponins, alkaloids and phenolic compounds in the berries of *P. dodecandra*. Thus, the secondary metabolites identified in the berries are all active antifungal compounds, which could imply that these secondary metabolites could be responsible for the antibacterial activity of the berries observed in the butan-1-ol extract of the berries [42].

From the dried roots of *Phytolacca dodecandra*, the three known olean-12-ene-dicarboxylic acids phytolaccagenin, phytolaccagenic acid and serjanic acid and two new genins, named as dodecandral and dodecandralol, have been isolated and characterized. The two new compounds have at C-20 an aldehyde function instead of a carboxyl group or its methyl ester [46].

The antirabies activity of both parts of plant extract in the doses of 300, 600 and 1000 mg/kg were compared with negative control based on the difference in survival rate and period (days) of group of mice challenged with rabies virus (CVS-11). The result showed that all doses of roots and 300 and 600 mg/kg doses of leaves of the plant extract didn't significantly($P > 0.05$) increase the survival period of mice compared to negative control group and significant ($P < 0.05$) survival period decrement was obtained compared to both positive control groups. However, 1000 mg/kg dose of leaves of the plant extract was significantly ($P < 0.05$) increased the survival period of mice as compared to their respective negative control group. The finding indicated the existence of some antirabies activity in extract of leaves of *P. dodecandra* at higher dose, for which further research is needed to elucidate its active ingredients [31].

The water extract of *Phytolacca dodecandra* has shown activity against a wide range of organisms. High antibacterial activity was shown against *Pseudomonas aeruginosa* and moderate activity against Bacillus subtilis, *Klebsiella pneumoniae, Serratia spp.* and *Salmonella typhi*. The plant has also been shown to possess antimicrobials agents like phenols, tannins, triterpenes, steroids and free amino acids [38].

The medicinal value of this plant could directly be attributed to one or a combination of two or more phytochemicals found in the plant to give a synergistic effect on the ailment been treated against. The antibacterial activity observed in this study could be directly attributed to the presence of important phytochemicals such as saponins, alkaloids and phenolic compounds [45].

CONCLUSION

The result from this study has shown that the aqua extract of *P. dodecandra* roots can inhibit both gram negative and gram positive bacteria *in vitro*. The pharmacologic mechanisms of action of the active ingredients must be studied and the active components can be considered for possible incorporation into conventional antibacterial drug production. The extract should also be tested for antifungal activity against some selected clinical isolates of fungal organisms.

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 Mr. Joel Ochieng Ondiek for his great assistance in the identification of the plant.

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CITE THIS ARTICLE AS:
In Vitro Antibacterial Activity of the Aqua Extract of *Phytolacca Dodecandra* Roots against Laboratory Strains of Selected Human Pathogenic Organisms, Anthoney Swamy T, Obey Jackie K, Miyogo Edwin O and Terer Erick, International Journal of Bioassays, 2015, 4 (05), 3903-3909.

Source of support: Nil
Conflict of interest: None Declared