Antimicrobial Effects Of Some Local Plants On The Growth Of *Agrobacterium tumefaciens* (Smith and Townsend), A Crown Gall Phytopathogenic Bacterium

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Abstract: *Agrobacterium tumefaciens* is a gram-negative, pathogenic bacterium causing crown gall disease on plants. The antimicrobial activities of aqueous leaf extracts of five plants: *Jatropha curcas*, *Moringa oleifera*, *Bauhinia monandra*, *Vernonia amygdalina*, *Azadirachta indica* were screened against *A. tumefaciens* using agar diffusion method. Fresh leaves of the test plants were collected, air dried and pulverized. Hundred grams of powdered leaves of each test plant was mixed with 200ml of distilled cold water at room temperature and left overnight, this was filtered and the filtrate served as extracts. The inhibitory effect of *A. indica* (0.20cm) extracts was highest at 5%. *J. curcas* (0.43cm) extracts inhibited most at 5%, while *V. amygdalina* extracts reduced the growth of *A. tumefaciens* at 5% by 0.46cm. Similarly, *M. oleifera* extracts was against *A. tumefaciens* at 5% by 0.70cm. *B. monandra* extracts was most inhibitive at 5% by 0.35cm.
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Introduction

Agrobacterium tumefaciens (Smith and Townsend) Conn, is an alpha-proteobacterium of the family *Rhizobaceae*, a gram-negative, rod-shaped and soil borne bacterium. It causes crown gall disease of plants roots and stems. *A. tumefaciens* infects plants through an open wound. It injects a section of its DNA into the host DNA and induces the plant to develop certain cell into a visible tumour called gall (Moore *et al*; 2008). This disease mostly affects dicotyledonous plants and causes economic damages to plants with great agricultural importance such as walnuts, tomatoes and roses. There is paucity of chemical pesticide for controlling *A. tumefaciens*. However, the ability of *A. radiobacter* to kill *A. tumefaciens* has been exploited by horticulturist (Ryder, 2010). In the recent years, there has been increased interest in the use of plant extracts for biological control of various pathogenic bacteria. Plant extracts and pure compound isolated from various plants have formed the bedrock of modern chemotherapy (Murti *et al*; 2010). It has been reported that Africa has as much as 300,000 medicinal plants (Dada, 2014). This finding is aimed at investigating the antibacterial effects of the aqueous leaf extracts of *M. oleifera, J. curcas, A. indica, B. monadra,* and *V. amygdalina* on growth of *A. tumefaciens*.

Materials And Methods

Location and Experimental site

Laboratory studies were conducted at Advanced Pathology Laboratory of the Institute of Agricultural Research and Training (IAR & T), Obafemi Awolowo University, Ibadan IAR & T is located to the North of Ibadan at latitude 7° 30N and longitude 3°45E. The altitude is 210m above sea level.

Sterilization of laboratory materials

Glass wares were washed in teepol detergent, mixed with water and allowed to dry. Erlenmeyer flask, beakers and pipettes were placed in aluminum foil while Petri dishes were placed in canisters and oven-sterilized at 160°C for at least 3 hours.

Inoculating needles, cork borers and scalpels were sterilized by flaming to red hot before use after dipping in 70% ethanol. The inoculating chambers (laminar flow hood) and all other working surface were sterilized by swabbing with 70% ethanol. Sterilization of media and distilled water were done in Erlenmeyer flask plugged with non-absorbent cotton wool and autoclaved at 121°C pressure for 15 minutes.

Preparation of sterile water

Nine ml of distilled water was pipetted into a clean dry test tube, plugged with cotton wool and wrap with aluminum foil. The test tubes were sterilized by autoclaving at 121°C for 15 minutes.

Preparation of media

Twenty eight grams of nutrient agar was poured into a clean dry one litre conical flask and 1000ml of distilled water was added to it. The flask was placed inside a water bath which was set at 90°C, the agar was allowed to dissolve.

The agar was distributed into Mac Cartney bottles and autoclaved at 121°C for 15 minutes. After cooling, 20ml of the media was poured into 9cm

sterile Petri dishes (Sterilin product, UK) which were then left to stand in a laminar flow cabinet for about 20 minutes to allow the nutrient agar to solidify.

Collection of plant samples

Fresh samples of plant were collected around the school premises. These were identified at the herbarium unit of Department of Plant Science, Ekiti State University. Air-drying was done for 3 weeks. It was later blended into powdery form with a blender and packed into a clean storing bottle until needed.

Extraction of plant extracts

Hundred grams of powdered leaves of each test plant was mixed with 200ml of distilled cold H_2O at room temperature and left overnight. Thereafter, this was filtered and the filtrate served as extract. Each extract was stored in a sterile bottle at 4^oC (Refrigerating time).

Isolation of surface bacteria contaminant on the plant extracts by serial dilution method

One ml of each of the plant extracts was taken using a syringe and dispensed into 9ml of sterile water. This was serially diluted. The final sample in the test tube was corked with cotton wool to avoid contamination.

Determination of Antibacterial activity

The antibacterial activity of the leaf extract was determined using agar well diffusion method. The molten nutrient agar was poured into a sterile Petri dish and allowed to cool to 45^0C, the bacterial inoculum was spread on the medium and mixed very well before it solidified. Wells were punched into the agar using 4m core-borer and the wells were filled with respective plant extract. Control experiment was set up using only the sterile distill water on the bacterial inoculums. The plates were incubated for 24 hours. The antibacterial activity was determined by measuring the diameter of the zone of inhibition using a ruler. The antibacterial potentials of different extracts were evaluated by comparing the zone of inhibition.

Preparation of standard antibacterial agent (streptomycin)

The molten nutrient agar was dispensed into a sterile Petri-dish and this was allowed to cool down to 45^oC, the bacterial inoculum was streaked on the medium. Wells were punched into the agar using 4mm cork borer and the wells were filled with a drop of streptomycin. The plates were incubated at 37^oC for 24 hours. The antibacterial activities were assayed by measuring the diameter of the zone of inhibition using metre rule.

Results

Surface contaminants associated with dried leaves of the test plants used in inhibiting the growth of *A. tumefaciens.*

The result in Table 1 shows that *B. cereus, B. subtilis, Corynebacterium, E. coli* and *S. aureus* were cultured as surface attachments from the various plants used in this study. The result revealed that *Bacillus subtilis* was present on all the plants (*J. curcas, V. amygdalina, A. indica, B. monandra*) except in *M. oleifera* extracts. Also, *B. cereus* and *B. subtilis* were found in *J. curcas, V. amygdalina* and *A. indica* extracts. Both *E. coli* and *S. aureus* were absent in *J. curcas* and *B. monandra.* However, *E. coli* was cultured from *V. amygdalina* and *A. indica*.

All the bacterial contaminants were associated with both *V. amygdalina* and *A. indica* except *Corynebacterium.* The result also showed that increased in the concentration of the plants extracts led to increase in the antibacterial activities of the extracts.

Table 1. Bacteria associated with dried leaves of the test plants used in inhibiting the growth of *A. tumefaciens*

Plant extracts	*B. subtils*	*B. cereus*	*Corynebacterium*	*E. coli*	*S. aureus*
J. curcas	+	+	+	-	-
V. amygdalina	+	+	-	+	+
A. indica	+	+	-	+	+
M. oleifera	-	-	+	-	+
B. monandra	+	-	+	-	-

+ = Present, - = Absent

All the plants extracts inhibited the growth of *A. tumfaciens* irrespective of the concentration. However, antibacterial activities of the concentrations of the plants extracts were significantly different. *B. monandra* (1.53%) at 20% concentration had the highest inhibitory activity on *A. tumefaciens*. *M. oleifera* at the lowest concentration of 5% inhibited the growth of *A. tumefaciens* (0.70%), compared to other plant extracts and the infective control (0.00%). *A. indica* showed the least inhibitory effect among the plant extracts (0.20%) at 5% concentration, streptomycin strongly inhibited the growth of *A. tumefaciens.*

Table 2: Inhibitory effects of the different plants extract on *A. tumefaciens*

Plant Extracts	5%	10%	15%	20%	LSD
J. curcas	0.43[c]	0.59[d]	0.77[d]	0.92[e]	0.52
V. amygdalina	0.46[c]	0.81[b]	1.03[b]	1.27[c]	0.29
A. indica	0.20[e]	0.48[e]	0.76[d]	0.90[e]	0.10
M. oleifera	0.70[b]	0.73[c]	0.90[c]	1.09[d]	0.30
B. monandra	0.35[d]	0.42[e]	0.65[e]	1.53[b]	0.31
Streptomycin	4.00[a]	4.00[a]	4.00[a]	4.00[a]	
Control	0.00[f]	0.00[f]	0.00[f]	0.00[f]	
LSD	0.18	0.19	0.18	0.32	

Value followed by the same letters is not significantly different from each other at p≤ *0.05*. (Fisher's LSD).

Discussion

This study elucidated the effectiveness of plant extracts on *A. tumefaciens*. Kilamuthu (2010) reported that the inhibitory activities of plant extracts depends largely on the concentrations of the extract, part of plant used and the strain of bacteria involved this might be responsible for the antibacterial effects of plants used for this study. Inhibition of the growth of *A. tumefaciens* by all the aqueous plant extracts has been reported (Afolayan, 2006; Mbaya, 2012; Dada, 2014). Ibikunle *et al.,* (2012) reported that antibacterial activity of *V. amygdalina* extracts may be due to the bioactive compounds like terpenoids, tannins, saponins, glycosides and alkaloids. Granesan (2008) reported antimicrobial potentials of *B. monandra*. The presence of flavonoid was attributed to antibacterial activity of *V. amygdalina* (Kumar *et al.,* 2005).

Conclusion

The investigation indicated variations in the bio-activities of the plants extracts used in inhibition of the bacterial growth. This showed that bioactive compounds present in different plants extracts may vary from one another. The outcome of this finding can play significant roles in containing phytobacterial infection on agricultural produce.

B. monandra showed the highest inhibitory effect on the bacterium. This may be as a result of flavonoids, saponins, tannins, alkaloids and steroids. Therefore, it can be used as a potential bactericide.

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