EVALUATION OF SIX MEDICINAL PLANT LEAF EXTRACTS AGAINST ACIDOVORAX CITRIULLI CAUSING BACTERIAL BLOTCH OF WATERMELON

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Abstract
Medicinal plants used in traditional medicine in Nigeria were tested for their antibacterial activities against *Acidovorax citrulli* the bacterial blotch pathogen of watermelon (*Citrullus lanatus*). Isolation of the pathogen was made from infected fruits on Nutrient Agar (NA) and pathogenicity was carried out on healthy watermelon fruits. The extracts from the leaves of these plants (*Entada africana, Vitex doniana, Lawsonia inermis, Azadirachta indica, Acalypha hispida and Nauclea latifolia)* were prepared using ethanol, cold and hot water. Phytochemicals analysis of the each extract was done using standard methods. Antimicrobial activities of each extract was evaluated at 50mg/ml using agar well diffusion techniques with bacterial inoculum of 2×10^6 cfu/ml. Control experiments were set up using sterile water as negative control and Gentamicin (10 ug/ml) as positive control. Minimum Inhibitory Concentration (M.I.C.) and Minimum Bactericidal Concentration (M.B.C.) were determined by a modification of the agar well dilution method. The results on phytochemical analysis revealed the presence of either one or more of the alkaloid, saponin, tannin, proanthocyanin, steroid and flavonoid in the six plants evaluated. Alkaloids, tannins and flavonoids were found in all the plant extracts. The weight of extract (g) obtained were significantly higher ($P<0.05$) with ethanol than cold and hot water extractants. The six plants exhibited varying degrees of antibacterial activity against the test organism. Ethanol extracts of all the plants gave the best inhibition of the bacteria with *A. indica and V. doniana* given the highest inhibition of 81% and 73% respectively against the pathogen. The ethanol extracts of *L. inermis*, *A. indica* and *N. latifolia* showed a broad spectrum of activity with MIC of 1.5mg/ml against the tested pathogen. Therefore, field trials of these six medicinal plants on the antibacterial activities against *Acidovorax citrulli* are recommended.

Keywords: Water melon, medicinal plant, *Acidovorax*, antibacterial, phytochemical.

Introduction
Watermelon (*Citrullus lanatus*) belongs to the family Cucurbitaceae. It is native to Kalahari desert of Africa but nowadays, it is also cultivated in tropical regions of the world (Edwards *et al*., 2003). It is used as a dessert fruit, a thirst quencher and relished by both man and his animals as a source of water in the very dry parts of Africa. Watermelon is a valued source of natural antioxidants with special reference to lycopene, ascorbic acid and citruline. These functional ingredients act as protection against chronic health problems like cancer insurgence and cardiovascular disorders (Fenko *et al*., 2009). Besides the presence of lycopene, it is a source of B vitamins, especially B1 and B6, as well as minerals such as potassium and magnesium (Huh *et al*., 2008). Watermelon contains phenolics quite comparable with that of other fruits (Kaur and Kapoor, 2001; Jaskani *et al*., 2005).

Bacterial fruit blotch or seedling blight caused by *Acidovorax citrilli* formerly *Pseudomonas pseudoalcaligenes* (Schaad *et al*., 1978 and Williems *et al*., 1992), is one of the diseases that

bedevil water melon (Latin, 2000; Roberts and Kucharek, 2006). The initial symptoms consist of irregular contour of yellow lesions or streaks on the upper surface of infected leaves. The lesions later turned rusty-coloured and were usually near the edges of the leaves. Infected leaves appeared water-soaked on the abaxial (underside) surfaces (Jett *et al*., 2002; Egel, 2007; Amadi *et al*., 2009).

A number of plants have been indicated to possess antimicrobial properties from their traditional medicinal uses (NCCLS, 2002). Neem (*Azadirachta indica)* has been used as repellent, insecticidal, antibacterial, antifungal, antifeedant, oviposition and growth inhibiting agent, and crop and grain protectant. *Lawsonia inermis has been* reported to have antibacterial, antifungal, antiparasitic, antiviral. *Vitex doniana* has been linked with antimicrobial properties (NCCLS, 2002). Hot aqueous extracts of *V. doniana* leaves are used in the treatment of stomach and rheumatic pains, inflammatory disorders, diarrhoea and dysentery. *Nauclea latifolia* is used in many African countries by traditional medical practioners for treatment of various ailments including bacterial diseases, malaria, gastrointestinal tract disorder, sleeping sickness, hypertension, toothache and septic mouth (Boye, 1990; Elujoba, 1995; Okwori *et al*., 2008). *Acalypha* spp. are used for the treatment of malaria, dermatolgical disorder, gastrointestinal disorders and known for their antimicrobial properties (Erute and Oyibo, 2008). Much work is done in the field of phytochemicals investigation of these plants; the chemical constituents isolated included napthoquinone derivatives, phenolic compounds, terpenoids, sterols, aliphatic derivatives, xanthones, coumarin, fatty acids, amino acids and other constituents (Boye, 1990; Iwu, 1993; Elujoba, 1995; Traore-Keita *et al.,* 2000; Okwori *et al*., 2008)

Plant derived medicines offer profound therapeutic benefits, more affordable treatment and relatively safer compare to the synthetic alternatives. On this note that this study investigated the i*n vitro* activities of Na*uclea latifolia, Vitex doniana, Lawsonia inermis, Azadirachta indica, Entada africana and Acalypha hispida* leaf extracts for biological control of *Acidovorax citrilli* the Bacterial blotch organism of watermelon (*Citrullus lanatus*) was investigated.

Materials and Methods

Collection of Plant materials

Plants used in this study *Entada africana, Vitex doniana, Lawsonia inermis, Azadirachta indica, Acalyppha hispida* and *Nauclea latifolia* (Table 1) were collected from wild from Lapai Local Government in Niger State Nigeria. Their botanical identifications were determined and authenticated with voucher numbers IBB/2017/06/001-006 respectively from the herbarium of Biology Department Ibrahim Badamasi Babangida University, Lapai Niger State Nigeria.

Table 1: Names and parts of medicinal plants collected for antimicrobial screening

Botanical name	Family	Common Name	Voucher No
Entada africana	Mimosaceae	Entada	IBB/2017/06/001
Vitex doniana	Verbenaceae	Black plum	IBB/2017/06/002
Lawsonia inermis	Lythraceae	Henna	IBB/2017/06/003
Azadirachta indica	Meliaceae	Neem	IBB/2017/06/004
Acalypha hispida	Euphorbiaceae	Red-hot cattail/Chenile plant	IBB/2017/06/005
Nauclea latifolia	Rubiaceae	African peach /pin cushion	IBB/2017/06/006

Preparation of Plant Extracts

The fresh leaves of the plants were washed using sterile distilled water (SDW) and air dried at room temperature (28 ± 2^{o}C) for four months. These materials were separately ground in porcelain mortal and later with a blender (model Mx – 49IN), sieved and stored in air tight bag.

Aqueous Leaf Extract

Twenty gram (20g) each of the samples were weighed and immersed in 250ml of cold SDW and another 20g of the sample also immersed in hot water (100^{o}C) in a conical flask for two hours. The extracts obtained were filtered twice through four layers of sterilized muslin cloth and a Whatman No 1 filter paper. The extracts were centrifuged at 5000rpm for 10min at 4^{o}C. To eliminate the solvent, the supernatants were evaporated using a 'Buuchi – R – 200' evaporator at 60^{o}C under vacuum. Extraction yield was calculated as percentage of the quantity of initial plant material (20g) (Okigbo, 2007).

$$\text{Yield (\%)} = \frac{\text{Mass of extract}}{\text{20.0g of powder}} \times \frac{100}{1}$$

The extracts were stored in refrigerator (4 ^{o}C) and used within 24hrs.

Ethanol Leaf Extract

Twenty gram (20g) of ground leaf with 100ml of 95% ethanol was added to a 500ml beaker. The extracts were filtered through four folds of sterilized muslin cloth. The supernatants were centrifuged at 500rpm for 10mins at 4^{o}C. To eliminate the solvent, the supernatant was evaporated using a 'Buuchi – R – 200' evaporator at 60^{o}C under vacuum. Extraction yield was calculated as percentage of the quantity of initial plant material (20.0g).

$$\text{Yield(\%)} = \frac{\text{mass of extract}}{\text{20.0g of powder}} \times \frac{100}{1}$$

The extracts were stored in refrigerator (4^{o}C) and used within 24hrs. The dried extract were exposed to ultra violet rays for 24hrs and check for sterility by plating on NA (Okigbo, 2007).

Phytochemical screening

Phytochemical test was conducted qualitatively to verify the presence or absence of secondary metabolites from the extract of the plants. A small portion of the extracts were subjected to phytochemical analysis using standard methods to test for presences of alkaloids, saponins, proanthocyanins, steroids, flavonoids, tannins and terpenoids (Sofowora, 1993; Okigbo & Igwe, 2007; Falodun *et al*., 2011).

Isolation and identification of Bacteria blotch

Isolations were made from infected leaves and fruits on Nutrient Agar (NA). Small sections (1mm diameter) of the infected leaves and fruits were cut into two separate 10-ml beakers. The fruit and leaf sections were surface-sterilized separately in Sodium hypochlorate ($NaOCl_3$) for 3 min and rinsed twice in sterile distilled water. The sections were blotted dry with sterile Whatman No. 1 filter paper and plated separately on sterile NA medium. Inoculated plates were incubated at room temperature (37±2°C) and colony development monitored. Morphological, physiological, and biochemical tests were carried out as described by Schaad *et al*. (1978) for proper identification of the pathogen.

Pathogenicity Tests

Scratching/wetting leaf abaxial surface with a suspension soaked gauze method was used. Inoculation was performed by scratching/wetting the abaxial surface of the first three leaves from three-week-old plants with a suspension-soaked gauze. Concentration of the bacterial suspension was adjusted to 10^6 CFU/mL with 0.005% Tween 20. Before and following inoculation the plants were covered with plastic bags for 24 and 48 hours respectively. The bacteria was re isolated and identified (Buso et al., 2004)

Preparation of the Test Bacteria

Fresh pure culture of test bacteria were made from the isolate cultures obtained on agar slants. With the aid of a sterile wire loop, colonies of fresh cultures of the bacterial isolates were picked and suspended on 5 ml nutrient broth in different labeled sterile 10 ml bijou bottles. One ml of the isolate from the broth was diluted in peptone water using the two-fold dilution. Since it is a fast growing organism, 0.02 ml of (10^{-4} to 10^{-10}) dilutions were plated on nutrient agar and incubated at 37^{o}C for 24 hrs. After 24 hrs, numbers of colonies observed were counted for the different diluents. The mean numbers of colonies were calculated to obtain the colony-forming unit per ml (cfu/ml) for the isolate.

Antimicrobial potential

One (1)ml of the standardised inoculum of the bacterial isolate was used in flooding sterile nutrient agar plates in the agar diffusion method for *in vitro* antimicrobial sensitivity test (Okwori *et al.*, 2008). A standardized inoculum of 2 X 10^6 cfu/ml was used for the inoculation of plates containing nutrient agar and incubated for 35 min at 37^{o}C. Three wells of 5.0mm diameter were cut on the inoculated plates using sterile cork borer. The cut agar discs were carefully removed by the use of sterile forceps. The well was filled with 1.5mg/ml concentration of plant extracts. Control experiments were set up by introducing SDW as negative control and Gentamicin (10 ug/ml) as positive control. Three replicates of each treatment were made; the plates were allowed to stand for one hour at (4^{o}C) in the refrigerator to allow for diffusion of the extracts into the NA. The plates were then incubated at (37 ± 2^{o}C) and the zone of inhibition measured after 24hrs. The zone of inhibition expressed in percentage was calculated (Okwori *et al.*, 2008; Adebola & Amadi, 2010; Ali *et al.*, 2011).

$$\text{Growth inhibition (\%)} = R_1 - R_2/R_1 \times 100$$

R_1 = length of colony growth with sterile distilled water

R_2 = length of colony growth with extract

Determination of Minimum Inhibitory Concentration (M.I.C.)

Minimum Inhibitory Concentration (M.I.C) was determined by a modification of the agar dilution method (Ali *et al.*, 2011). The extracts were sterilized using corning sterile syringe filter (0.2Gm pore size). Ten (10) ml of 1.5mg/ml of the extract concentrations 1.5mg/ml was incorporated into 10ml of double strength molten agar at 50^{o}C and aseptically poured into Petri-plates. After setting, 6mm sterile Whatmann filter paper discs in duplicates were aseptically applied to the surface of the set agar containing the various extract concentrations. 10ul of each standardized inoculum (10^6cfu/ml) was then spot-inoculated onto each disc and allowed to diffuse for 25 mins before incubating at 37^{o}C for 18 hrs. The first and lowest concentration that showed no visible growth of the inoculated tested organism was recorded as the M.I.C. of the extract for the test organism.

Determination of Minimum Bactericidal Concentration(M.B.C.)

All inoculated paper discs showing no visible growth from the M.I.C. determination were transferred to 5ml of sterile Nutrient Broth containing 5% Tween 80 to neutralize the effect of the extracts and incubated for another 18 hrs. The discs from the lowest concentration of each plant extract that showed no visible growth (cloudiness) was taken as the M.B.C. of that plant extract against the test organism (Ali *et al*., 2011).

Results

Isolation and pathogenicity test

The characterization and pathogenicity tests results confirmed that the bacteria isolated was *A. citrulli* and authenticated as the causative organism of bacteria blotch of water melon.

Yield of the crude extract

From the results on yield of the crude extracts obtained from the six plants (Table 2), ethanol gave the highest percentage yield of extract while cold water gave the least. The weights of the extract (g) produced in all the extracts were significantly different (P<0.05). In *E. africana*, the percentage yield in cold water was 5% followed by hot water 12% and ethanol 15%. This trend was obtained in other plant extracts screened. The weight of extract (g) obtained from various extractants were significantly different (P<0.05), however, in *E. africana*, weights obtained from hot water (2.4g) and ethanol (3.0g) were not significantly different (P< 0.05). The weights of the extract obtained from cold and hot water extract of *A. hispida (*3.5 and 3.7g respectively*)*were not significantly different. In *V. doniana, L. inermis, A. indica* and *N. latifolia,* the weights of the extracts produced by the extractants were significantly different(P<0.05).

Table 2: Percentage yield of the crude extract of six medicinal plants screened

Plant material	Wt. of extract(g)			Percentage yield of extract(%)	
	CW	HW	ET	CW	HW
ET					
E. africana	$1.00_{\mathbf{a}}^{\mathbf{a}}$	$2.40_{\mathbf{a}}^{\mathbf{b}}$	$3.00_{\mathbf{a}}^{\mathbf{b}}$	5.00_{a}^{a}	12.00_{a}^{b}
15.00_{a}^{c}					
V. doniana	$1.50_{\mathbf{a}}^{\mathbf{a}}$	$3.60_{\mathbf{b}}^{\mathbf{b}}$	$5.20_{\mathbf{c}}^{\mathbf{c}}$	7.50_{b}^{a}	18.00_{b}^{b}
26.00_{c}^{c}					
L. inermis	$2.00_{\mathbf{a}}^{\mathbf{a}}$	$2.60_{\mathbf{a}}^{\mathbf{b}}$	$3.20_{\mathbf{a}}^{\mathbf{c}}$	10.00_{c}^{a}	13.00_{a}^{b}
16.00_{a}^{c}					
A. indica	$2.60_{\mathbf{b}}^{\mathbf{a}}$	$4.30_{\mathbf{c}}^{\mathbf{b}}$	$5.30_{\mathbf{c}}^{\mathbf{c}}$	13.00_{c}^{a}	21.50_{c}^{b}
26.50_{c}^{c}					
A. hispida	$3.50_{\mathbf{b}}^{\mathbf{a}}$	$3.70_{\mathbf{b}}^{\mathbf{a}}$	$4.20_{\mathbf{b}}^{\mathbf{b}}$	17.50_{d}^{a}	18.50_{c}^{a}
21.00_{b}^{b}					
N. latifolia	$2.40_{\mathbf{b}}^{\mathbf{a}}$	$3.30_{\mathbf{b}}^{\mathbf{b}}$	$5.10_{\mathbf{c}}^{\mathbf{c}}$	12.00_{a}^{a}	16.50_{c}^{b}
25.50_{c}^{c}					

*Numbers followed by different letters on the same row or column differ significantly at P < 0.05
Key: CD= Cold water HW= Hot water ET= Ethanol

Phytochemical screening
Phytochemical analysis (Table 3) revealed the presence of either one or more of the alkaloid, saponin, tannin, proanthocyanin, steroid and flavonoid in the six plants screened. Alkaloids, tannins and flavonoids were found in all the plant extracts. The results showed that with the exception of *E . africana* and *A. indica* proanthocyaninwas present in all the extracts. Steroid was also present in all thr plant extracts except *V.doniana* and *E. africana.* Saponin was not found in *Acalypha hispida*. Only *L. inermis* contained all these phytochemicals.

Antimicrobial activity
Antimicrobial activity of different solvent extracts on the test organism (Table 4), was in evidence by different zone of inhibitions produced. The susceptibility pattern of the test organisms to the extract in this study was not uniform (Table 4), ethanol extracts of each plants gave the highest zone of inhibitions. In *E . africana*, ethanol extract produce 66% zone of inhibition followed by cold water extract(56%) and least by hot water extract (52%) while in *N. latifolia*, it was 72% in ethanol, 58% in hot water and 47% in cold water extracts.

Table 3: Phytochemical constituents of six medicinal plants screened Phytochemical constituents

Species	Alkaloids	Saponins	Tannin	Proanthocyanins	Steroids	Flavonoids
Entadra africana	+	+	+	-	-	+
Vitex doniana	+	+	+	+	-	+
Lawsonia inermis	+	+	+	+	+	+
Azadirachita indica	+	+	+	-	+	+
Acalyppha hispida	+	-	+	+	+	+
Nuaclea latifolia	+	+	+	+	-	+

Key: += Present - = Absent

The results of the minimum inhibitory concentrations (MIC) of the extracts on the test organism are shown in Table 5. There was significant difference (P<0.05) in MIC produced by ethanol, hot and cold water extracts of *V. doniana, L. inermis, Entada africana* and *Acalypha hispida.* But in *N. latifolia* and *A. indica* there was no significance difference. Ethanol and hot water extracts showed a more potent inhibitory effect against the pathogen with MIC value of 1.5mg/ml. In hot and cold water extracts the MIC produced was not significantly different (P<0.05) except in *V. doniana* and *L. inermis*. The MIC in ethanol extracts of all the plants varied from 0.5mglml to 5mg/ml were the same in *L. inermis* and *V. doniana*, but were significantly different (P<0.05) from cold water extracts which was higher in both plant extracts. However, the same MIC value of 1.5mg/ml was obtained in all the extractants of *A. indica* and *N. latifolia*.

From the results of MBC of the screened crude extracts (Table 6), ethanol extracts gave the least MBC while cold extract gave the highest comparatively. In *L. inermis, A. indica* and *N. latifolia,* MBC were not significantly different (P<0.05)between hot water and ethanol extracts.

Table 4: Antimicrobial activity of different solvent extract on *Acidovorax citrilli* (zone of inhibition expressed in percentage)

Plant species	Colony growth Inhibition (%)		
	Cold Water	Hot water	Ethanol
Entada africana	56	52	66
Vitex doniana	48	67	73
Lawsonia inermis	43	48	64
Azadirachta indica	57	64	81
Acalypha hispida	39	43	67
Nauclea latifolia	47	58	72

Table 5: Minimum Inhibitory Concentration (M.I.C.) of the crude extracts (mg/ml) against *Acidovorax citrilli*

Plant species	Cold extract*	Hot extract*	Ethanol extract*
Entadra Africana	15.0b	15.0b	1.5a
Vitex doniana	20.0b	5.0a	5.0a
Lawsonia inermis	5.0b	1.5a	1.5a
Azadirachita indica	1.5a	1.5a	1.5a
Acalypha hispida	15.0b	15.0b	5.0a
Nuaclea latifolia	1.5a	1.5a	1.5a

*Numbers followed by different letters on the same row differ significantly at P < 0.05

Table 6: Minimum Bactericidal Concentration (M.B.C.) of the Crude Extracts (mg/ml)against *Acidovorax citrilli*

Plant species	Cold extract*	Hot extract*	Ethanol extract*
Entadra Africana	20.0c	15.0b	5.0a
Vitex doniana	50.0c	20.0b	15.0a
Lawsonia inermis	20.0b	1.5a	1.5a
Azadirachita indica	15.0b	1. 5a	1.5a
Acalypha hispida	20.0b	35.0c	15.0a
Nuaclea latifolia	15.0b	1.5a	1.5a

*Numbers followed by different letters on the same row differ significantly at P < 0.05

Discussion

The results showed that the yield of the extracts differ with preference to the plants and the solvent (extractants) used. The highest yield was obtained from ethanol extracts followed by hot water extracts. This may be because ethanol is an organic solvent and will dissolve organic compounds better than water as earlier reported by Okigbo and Igwe, (2007).

The phytochemical constituents obtained from the plant extracts in this study namely; alkaloids, saponins, tannins, proanthocyanins, steroids, and flavonoids had earlier being reported in some of these plants(Okigbo and Igwe, 2007). The presence of alkaloids in *N. latifolia* extract was not in agreement with the earlier report of Nworgu *et al.* (2008), probably due to environmental variation in the areas where the plants were collected. Only *L. inermis* contained all the phytochemicals tested has earlier reported by Nworgu *et al.*(2008). The antimicrobial activities of the plant extracts on *A. citrulli* also revealed that all the six plant extracts had significant

positive effect on growth inhibition of the pathogen. Probably, the alkaloid present in them may be toxic to the pathogen and thus, affecting their physiological pathways. The high antimicrobial properties or chemical potency of these phytochemicals justified inclusion of some of the plants that contain them in herbal medicine by the indigenous people (Okigbo and Igwe, 2007). The result showed that the pathogen was more susceptible to alcoholic extracted than aqueous extracts probably because ethanol has high phytochemical extraction capability and dissolves organic compounds better.

The botanical samples screened in this study exhibited varying degrees of antibacterial activity against the pathogen; this was also reported by Bashir *et al.* (2011). The antimicrobial activity was observed to increased with increase in concentration of botanical extracts. This might be as a result of the phytochemicals constituents of these plants. Owoyele *et al*. (2005) reported that the antimicrobial activity of plants is associated with the presence of some chemical components such as phenols, tannins, alkaloids, steroids and flavonoids. Okwori *et al*. (2008) and Umeh *et al.* (2005) also reported that the antibacterial constituents of plant are preferentially concentrated in their leaves. The ethanolic extracts produced a high inhibitory effect against *A. citrulli* with MIC values ranges from 1.5mg/ml to 5.0mg/ml. This confirmed the earlier report that alcohol extracts possessed broad spectrum of activity against both bacteria and fungi (Ali *et al.,* 2011). The MIC and MBC values were equal for hot water and ethanol extracts of *L. inermis, A. indica* and *N. latifolia* possibly indicating that the extracts could be bactericidal to the organism. For *V. doniana* and *A. hispida*, the MBC is greater than corresponding MIC possibly because the extracts were merely bacteriostatic as suggested by Ali *et al.* (2011).

Antimicrobial activity of the *V. doniana* extract could be attributed to the presence of phenolic compounds that have been liked with antimicrobial properties (Elujoba, 1996; NCCLS, 1995).The low MIC values confirm high activity of the extract at low concentrations

References

Adebola, M. O., & Amadi, J. E. (2010). Screening three *Aspergillus* species for antagonistic activities against the cocoa black pod organism(*Phytophthora palmivora*). *Agric. And Biology Journal of North America*, 1(3), 362-365.

Amadi, J. E., Adebola, M. O., & Eze, C. S. (2009). Isolation and identification of a bacterial blotch organism from watermelon (*Citrullus lanatus* (Thunb.) Matsum. and Nakai). *African Journal of Agricultural Research,* 4(11), 1291-1294.

Ali, A. I., Ehinmidu, J. O., & Ibrahim, Y. K. E. (2011). Preliminary phytochemical screening and antimicrobial activities of some medicinal plants used in Ebira land. *Bayero Journal of Pure and Applied Sciences*, 4(1), 10 – 16.

Bashir, M., Yusuf, I., & Kutama, A. S. (2011). In-vitro studies on the sensitivity of Staphylococcus aureus to some ethno-medicinal preparations sold around Kano, Nigeria. *Bayero Journal of Pure and Applied Sciences,* 4(1), 22 – 25.

Boye, G. L. (1990). Studies on antimalarial action of *Cryptolepsis sanguinolenta* extract. Proc.Int. Syp. On East – West Med. Seoul, Korea: 243 - 251

Buso, G. S. C., Nass, L. L., Marques, A. S. A., Lopes, C. A., & Buso, J. A. (2004). Avaliação de genótipos de melão, visando identificar fontes de resistência a Acidovorax avenae subsp. citrulli.

Brasília, D. F., (nd) Embrapa Recursos Genéticos e Biotecnologia. Comunicado Técnico 116

Edwards, A. J., Vinyard, B. T., Wiley, E. R., Brown, E. D., Collins., J. K., & Perkins-Veazie, P. *et al*., (2003). Consumption of watermelon juice increases plasma concentrations of lycopene and β-carotene in humans. *Journal of Nutrion,* 133, 1043-1050.

Egel, D. (2007). Fruit diseases of muskmelon and watermelon. *The New Agriculture Network Newsletter,* 4(7). www.newag. msu.edu/issues07/8-15htm # 1

Elujoba, A. A. A. (1995). Female infertility in hands of Traditional Birth attendants in South – West Nigeria. *Fitoterapia,* 66 (3), 239-248.

Elujoba, A. A. (1996). Standardization of Phytomedicines Proceedings on an international workshop on commercial production of an indigenous plant, Lagos. Nigeria. p19.

Erute, M. O., & Oyibo. E. A. (2008). Effects of three plants extract(Occimum gratissimum, Acalypha wilkesianab and A. macrostachya) on post harvest pathogens of Persia Americana. *Journal of Medicinal Plants Resh,* 2,311-314.

Falodun, A., Osakue, J., Uzoekwe, A. S., & Qiu, S. (2011). Phytochemical and anticancer studies on the ten medicinal plants used in Nigeria. *Bajopas,* 4(1), 36-39.

Fenko, A, Schifferstein, H. N., Huang, T. C., & Hekkert, P. (2009). What makes products fresh: The smell or the colour? *Food Qual Prefer,* 20,372-9.

Huh, Y. C., Solmaz, I., & Sari, N. (2008). Morphological characterization of Korean and Turkish watermelon germplasm. In: Pitrat M (ed): Cucurbitaceae 2008. Proceedings of the IXth EUCARPIA meeting on genetics and breeding of Cucurbitaceae, Avignon (France), 327-33.

Iwu, M. M. (1993). Handbook of African medicinal plants. CRC Press Inc. USA. PP 219- 221

Jett, L. W., Baker, T. P., & Corwin, B. (2002). Watermelon bacterial fruit blotch. University of Missouri Extension. www.extension.missouri.edu/explore/agguides/pests/ipm1011.htm

Kaur, C., & Kapoor, H. C. (2001). Antioxidants in fruits and vegetables–the millennium's health. *Int J Food Sci Technol,* 36, 703-25.

Latin, R. X. (2000). Bacterial fruit blotch of cucurbits. Purdue University, West Lafayette, IN 47907.

National Committee for Clinical Laboratory Standard (2002). Reference method for broth dilution antifungal susceptibility testing of filamentous fungi. Approved by standard M38-A. National Committee for Clinical Laboratory Standards,Wayne, PA

National committee for Clinical laboratory Standards (NCCLS). (1995). Performance Standards for antimicrobial Susceptibility testing, 15(14), 100 – 56

Nworgu, Z. A., Onwukaeme, D. N., Afolyan, A. J., Ameachina, F. C., & Ayinde, B. A. (2008). Preliminary studies of blood pressure lowering effect of *Nuclea latifolia* in rat. African *Journal of Pharmacy and Pharmacology*, 2(2), 37-41.

Oladunmoye, M. K. (2006). Comparative evaluation of antimicrobial activities and phytochemical screening of two varieties of Acalypha wilkesiana. *Trends in Applied Science Research,* 1, 538-541.

Okigbo, R. N., & Igwe, D. I. (2007). Antimicrobial effects of *Piper guineese* and *Phyllantus amarus* 'Ebe-Benizo' on *Candida albicans* and *Streptococcus faecalis. Acta Microbiologica et Immuunologica Hungarica,* 54, 353-366.

Okwori, A. E., Okeke, C. I., Uzoechina, A., Etukudoh, N. S., Amali, M. N., Adetunji, J. A., & Olabode, A. O. (2008). The antibacterial potentials of *Nuclea latifolia*. *African Journal of Biotechnology,* 7(10),1394-1399.

Okwori, A. E. J., Dina, C. O., Junaid, S. A., Okeke, I. O., Adetunji, J. A.,. & Olaode, A. O. (2007). Antimicrobial activities of *Ageratum conyzoides* on some selected bacterial pathogens. *Internet Journal Microbiol*, 4(1), 22-30.

Onocha, P. A., & Olusany, T. O. B. (2010). Antimicrobial and anthelmimtic evaluation of Nigerian Euphorbiaceae plants 3. *Acalpha wilkesiana African Scientist,* 11(2), 85-89.

Owoyele, V. B., Adeyemi, F. M., & Soladoye, A. O. (2005). Effect of aqueous leave extract of *Ocimum gratissimum* (sweet basil) on alloxan induced diebetic rats. *Pharmocognosy Magazine,* 1(2), 142-146.

Roberts P, KucharekT (2006). Florida Plant Disease Management Guide: *Watermelon*. http://watermelons.ifas.ufl.edu/diseases.htm

Schaad, N. W., Sowell, Jr G., Goth, R. W., Colwell, R. R., & Webb, E. (1978). Pseudomonas pseudoalcaligenes subsp. citrulli subsp. nov. *International Journal of Systematic Bacteriology,* 28,117-125.

Sofowora, A. (1993). *Medical plants and traditional medicine in Africa. 2nd ed.* Ibadan: Spectrum Books Ltd. Pp. 71-73

Taore-Keita, F., Gasquet, M., Giogio, C., Ollivier, E., Delmas, F., & Keita, A. (2000). Anti-malaria activity of four plants used in traditional medicine in Mali. *Phytother Res.,* 14, 45-47.

Umeh, E. U., Oluma, H. O. A., & Igoli, J. O. (2005). Antibacterial screening of four local plants using an indicator – based microdilution technique. *African Journal Tradit.*, 2(3), 238-243.

Willems A., M. Goor, S. Thielemans, M. Gillis, K. Kersters K and Jde Ley. 1992. "Transfer of several phytopathogenic *Pseudomonas*species to *Acidovorax* as *Acidovorax avenae* subsp. *avenae* subsp. nov., comb. nov., *Acidovorax avenae* subsp. citrulli, *Acidovorax avenae*subsp. *cattleyae*, and *Acidovorax konjaci*." *International Journal of Systematic Bacteriology,* 42(1), 107-119.