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The effect of autoclaving and membrane filtration on the antimicrobial activities of *Alchornea cordifolia* leaf extract

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Autoclaved and membrane filtered sets of the ethanolic leaf extract of *Alchornea cordifolia* (Schum and Thonn) Muell. Arg were screened against reference bacterial strains using the cup-in-plate agar diffusion and the agar dilution techniques. The zones of inhibition produced by the unsterilized extract and the autoclaved extract were not significantly different. The membrane filtered extract produced smaller zones of inhibition (14 – 16mm) of the bacteria than the unsterilized (14 – 22mm) and autoclaved extracts (12 – 22mm). The minimum inhibitory concentrations of the filtered extract against *Bacillus subtilis* and *Escherichia coli* was more than double those of the unsterilized and autoclaved extracts. The results suggest that autoclaving can be a useful method of sterilizing the ethanolic extract of *Alchornea cordifolia* leaves.

Keywords: *Alchornea cordifolia*; antimicrobial activity; sterilization; autoclaving; membrane filtration.

Introduction

Antimicrobial resistance is one of the biggest challenges facing global public health and this is a matter of global concern because many species and strains of bacteria that are pathogenic to humans have developed resistance to both the well established and newer antibiotics (Livermore, 2003). Most reports suggest that the main force behind this emergence of antimicrobial drug resistance is the inappropriate use of antibiotics by man during the past few decades (Alanis, 2005) but there is also evidence for the epidemic spread of drug-resistant bacteria as a contributing factor (Livermore, 2003). One of the solutions that have been put forward against the problem of antibiotic resistance is the search for alternative sources of antimicrobial agents especially from plants (Cordell, 2000). Many plants have been found to possess various degrees of antimicrobial activity using methods of assessment such as agar diffusion techniques, agar dilution and the broth dilution methods. One problem associated with the broth dilution method of testing plant extracts is the influence of microbial contaminants usually present in an unsterile extract which may make it difficult to read results accurately. This problem can be overcome if sterile plant extracts are used in the tests for antimicrobial activities. There is however paucity of data on the effect of different sterilization methods on the antimicrobial activity of plant extracts.

Alchornea cordifolia (Schum and Thonn) Muell. Arg. leaf has been reported to possess antimicrobial activity (Adeyemi *et al*., 2008) and its spectrum of activity has been shown to include the Gram negative bacteria, the Gram positive bacteria as well as yeasts (Edema and Osarumwense, 2007). Adeshina *et al*., (2011) reported the effects of hot air sterilization method and moist heat sterilization on the antimicrobial property of *A. cordifolia* leaf extracts. This study was undertaken to compare the effect of autoclaving with that of membrane filtration on the antimicrobial activities of *A. cordifolia* leaf extract.

Materials and Methods

Collection and extraction of plant material

Alchornea cordifolia (Schum and Thonn) Muell. Arg. leaves were collected in April 2012 at the Obafemi Awolowo University (O.A.U.) Campus, Ile-Ife, Nigeria. The leaves were air dried at room temperature and ground into powder. A Soxhlet extractor was used to extract 620g of the powdered leaf exhaustively with ethanol. After the extraction, the extract was concentrated *in vacuo* and freeze-dried to yield 138.3g of dried powder.

Sterilization of test extract

Three sets of preparations from the same batch of *A. cordifolia* leaf extract each at concentrations 12.5mg/ml, 25mg/ml, 50mg/ml, 100mg/ml and 200mg/ml in sterile water were prepared for the evaluation.

A set was sterilized in a portable autoclave at 121^{0}C for 20 minutes while a second set was filtered through a sterile cotton cloth for clarification before passage through a 0.45µm membrane filter. The third set was the untreated samples.

Test organisms:
Reference strain of *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* NCTC 8236, *P. aeruginosa* ATCC 10145 and *Escherichia coli* ATCC 25922 were obtained from stocks of culture collections maintained in our laboratory.

Determination of Zones of Inhibition:

Colonies of 18 h nutrient agar culture of each test microorganism were suspended in sterile water and the turbidity was adjusted to approximately 10^6cfu/ml using the McFarland Standard as reference. Into each test plate was poured molten sterile nutrient agar (20 ml) which were allowed to set and harden. Sterile cotton tipped applicators were used to swab duplicate agar plates with each test organism and allowed to acclimatize for 15 minutes. A sterile cork borer (diameter 8mm) was then used to bore cups equidistant to one another into the agar plate. A drop of the molten agar was used to seal the bottom of each hole to prevent the test extracts from sipping beneath the agar. 4 drops of each of the prepared concentrations of the test extract (12.5mg/ml, 25mg/ml, 50mg/ml, 100mg/ml and 200mg/ml) were then introduced into respective holes for each concentration. Sterile water was used as the negative control while ciprofloxacin disc (5µg) was used as a reference antimicrobial agent. The plates were left at room temperature for one hour to allow for the diffusion of the extract and antibiotic before incubating at 37°C for 18 h. At the end of the incubation period, the zones of inhibition were measured in millimeter and expressed as the mean of the plates.

Determination of Minimum Inhibitory Concentrations (MICs) of the extract:
MICs were determined using the agar dilution method (CLSI, 2007). A stock suspension of 500 mg/ml of extract in water was prepared. From this agar plates containing 50, 40, 20, 10, 5 and 2.5 mg/ml of *Alchornea cordifolia* extract were prepared. Plates containing chlorocresol (BDH) 0.125, 0.0625, 0.03125 and 0.015625 mg/ml were similarly prepared to serve as controls. The test microorganisms were grown overnight in nutrient agar plates (Oxoid, England) at 37^{o}C. Two to three colonies of each microorganism were suspended in sterile water and diluted to a final density of 2 x 10^6 cfu/ml using the Mcfarland Standard as a reference. Duplicate *Alchornea cordifolia* agar plates were then challenged with the test microorganism suspensions using a multi-inoculator device that deposits 2µl of each microorganism suspension on the plates and incubated at 37^{o}C for 72h. All plates were observed for growth and the minimum dilution completely inhibiting the growth of each test organism was taken as the MIC.

Statistical analysis:
Results were expressed as mean ± SD (standard deviation). The significance of differences between values was determined using Student t-test**.** P-values less than 0.05 were considered statistically significant.

Results and Discussion

The zones of inhibition (mean of duplicate plates) produced by the different sets of sterilized and the unsterilized extract against the test organisms as well as the minimum inhibitory concentrations of the extracts are as shown in Tables 1 and 2 respectively.
These results showed that *A. cordifolia* leaf extract sterilized in the autoclave was slightly more active than the unsterilized extract against the test microorganisms. The difference was however not statistically significant. This suggests that the active principles in the extract are stable to a temperature of 121^{o}C for up to 20 minutes which was the condition for autoclaving in this study. The active principles in *A. cordifolia* has been reported to belong to classes of compounds such as tannins, alkaloids, anthraquinones, saponin, glycosides and flavonoids and these compounds include gallic acid, ellagic acid, protocatechic acid, quercetin, hyperin and guaijaverin (Okwu *et al*., 2010; Adeyemi *et al*., 2008; Udobi *et al*., 2008; Adeshina *et al*., 2007; Lewis and Ausubel, 2006; Palombo, 2006; Agbor *et al*., 2004). Some of these compounds have been reported to be hydrolysable at high temperatures either into more active compounds or less active compounds (Song and Milner 2001). As reported by Kim *et al.,* (2010), the antimicrobial capacity of tannic acid was enhanced by exposure to temperatures between 105^{o}C and 150^{o}C which produces gallic acid and pyrogallol with pyrogallol showing higher antimicrobial activity than gallic acid (Kim *et al*., 2010). The results obtained in this study seem to suggest that any loss in the antimicrobial activity of a compound which had undergone hydrolysis is being compensated by the increase in activity of some other compounds as a result of the high temperature. This needs to be investigated further by testing each of the isolated compounds of *A. cordifolia* leaf extract.
The extract sterilized by passage through a bacteria proof membrane filter, on the other hand, showed significantly lower inhibition of the test microorganisms than the unsterilized and the autoclaved extract. A possible reason for this observation may be because the antimicrobial principles in the extract are not all completely soluble in water and the use of the filters

results in the removal of particles which are bigger than the pore size of the filters leading to a reduction in the concentration of the active principle in the filtrate. This lower concentration will definitely reduce the potency of the extract against any test microorganisms. The minimum inhibitory concentrations of the different extract also followed a similar pattern as the zones of inhibition. A much higher concentration of the filtered extract was needed to inhibit the susceptible test microorganisms. Adeshina *et al*., (2011) compared the effects of hot air sterilization at 100 ^{o}C in an oven for 30 minutes with steam sterilization at 121 ^{o}C for 15 minutes on the antimicrobial property of *A. cordifolia* methanolic leaf. They reported the loss of antimicrobial property in *A. cordifolia* methanolic leaf extract treated using hot air while the sample treated using steam sterilization retained its activity. The findings in this study therefore corroborate the earlier result and in addition showed that membrane filtration may not be the choice of sterilization of *A. cordifolia* leaf extract.

In conclusion, the results of this study have shown that the antimicrobial activity of *A. cordifolia* leaf extract is not reduced or neutralized by heating at 121 ^{o}C for 20 minutes, an indication that autoclaving is a suitable choice for the sterilization of *A. cordifolia* leaf extract.

Table 1: Zones of inhibition of test microorganisms by unsterilized, autoclaved and filtered extracts.

Concentration of extract (mg/ml)	**Organisms / Zones of inhibition (mm)**			
	B. subtilis NCTC 8236	*S. aureus* ATCC 25923	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 10145
U12.5	-	-	-	-
U25	14 ± 0.35	12 ± 0.0	-	-
U50	15.5 ± 0.35	13 ± 0.0	-	-
U100	19 ± 0.0	17.5 ± 0.35	-	10.5 ± 0.35
U200	22± 0.0	20 ± 0.0	13.5 ± 0.35	12.5 ± 0.35
A12.5	-	-	-	-
A25	12 ± 0.0	10 ± 0.0	-	-
A50	14.5 ± 0.35	11.5 ± 0.35	-	-
A100	20 ± 0.0	13.5 ± 0.35	-	10 ± 0.0
A200	22 ± 0.0	17.5 ± 0.35	13.5 ± 0.35	12.5 ± 0.35
F12.5	-	-	-	-
F25	-	-	-	-
F50	-	10 ± 0.0	-	-
F100	14 ± 0.0	13 ± 0.0	-	-
F200	16 ± 0.0	16 ± 0.0	-	10 ± 0.0
Ciprofloxacin(5µg)	15± 0.0	20 ± 0.0	25 ± 0.0	25 ± 0.0

Key- U: Unsterilized; A: Autoclaved; F: Filtered

Table 2: Minimum inhibitory concentration of unsterilized, autoclaved and filtered extracts against test microorganisms

Extract	Microorganisms / Minimum Inhibitory Concentration (mg/ml)			
	B. subtilis NCTC 8236	*S. aureus* ATCC 25923	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 10145
Unsterilized	≤ 5	≤ 5	≤ 20	≤ 20
Autoclaved	≤ 5	≤ 5	≤ 20	≤ 20
Filtered	≤ 20	≤ 10	>50	≤ 20

REFERENCES

Adeshina GO, Akut S, Onaolapo JA, Ehinmidu JA, Odama LE (2011). Effect of heat on the antimicrobial activity of A*lchornea cordifolia* leaf extracts**.** *International Journal of Applied Science and Technology* 1(5): 227-232.

Adeshina GO, Onaolapo JA, Ehinmidu JO, Odama LE, Kunle OF (2007). Phytochemical and toxicologic activity of the leaf extracts of *Alchornea cordifolia* (Schum and Thonn) Muell. Arg. (Euphorbiaceae). *Nig. J. Pharm. Res*. 6(1): 19-24.

Adeyemi A, Omonigbehin E, Smith S, Odusola O, Sobande J (2008). Antibacterial activity of extracts of *Alchornea cordifolia* (Schum and Thonn) Muell. Arg.*, Boerhavia diffusa* (L) and *Bridellia micranthal* (Hoscht) Baill. used in traditional medicine in Nigeria on *Helicobacter pylori* and four diarrhoeagenic bacterial pathogens**.** *Afr J Biotech* 7 (20): 3761-3764.

Agbor C, Leopold T, Jeanne NY (2004). The antidiarrhoeal activity of *Alchornea cordifolia* leaf extract. *Phytother. Res*. 18(11): 873-876.

Alanis AJ (2005). Resistance to Antibiotics: are we in the post-antibiotic era? *Archives Med Res*. 36:697-705.

Cordell GA (2000). Biodiversity and drug discovery — a symbiotic relationship. *Phytochemistry* 55(6): 463–480.

Clinical and Laboratory Standards Institute (2007). Performance standards for antimicrobial disk susceptibility tests. Approved standard. 9th ed. Document M2–A9. Wayne (PA).

Edema MO, Osarumwense OP (2007). Preliminary Studies on the Phytochemical and Pharmacological Screening of *Alchornea Cordifolia***.** *Nigerian Journal of Chemical Research* 12: 51-57.

Kim T, Silva J, Jung Y (2010). Enhanced functional properties of tannic acid after thermal hydrolysis. *Food Chemistry* 126: 116-120.

Lewis K, Ausubel FM (2006). Prospects for plant derived antibacterials. *Nat. Biotechnol*. 24(12): 1504-1507.

Livermore DM (2003). Bacterial resistance: origins, epidemiology, and impact. *Clin Infect Dis* 36(Suppl 1):S11-23.

Palombo EA (2006). Phytochemicals from traditional medicinal plants used in the treatment of diarrhoea: mode of action and effects on intestinal function. *Phytother. Res*. 20(9): 717-724.

Song K, Milner JA (2001). The influence of heating on the anticancer properties of garlic. *Journal of Nutrition*. 131(3s):1054S-1057S.

Udobi CE, Onaolapo JA, Agunu A (2008). Antibacterial activities and bioactive components of the aqueous fraction of the stem bark of *Parkia bigblobosa* (JACQ) (Mimosaceae). *Nig. J. Pharm. Sci.* 7(1): 49-55.