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# Phytochemical Screening and Stability Studies on the Antimicrobial Activities of the Leaf Extracts of *Alchornea cordifolia* (Schum. & Thonn.) Muell. Arg. (Euphorbiaceae).

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**Abstract**

The phytochemical screening of the water, methanol and ethyl acetate extracts of *Alchornea cordifolia* leaf using standard biochemical methods revealed the presence of tannins, saponin, flavonoid, alkaloid, glycoside and phenol. The antimicrobial activity of the water, methanol and ethyl acetate extracts of the *Alchornea cordifolia* leaf against *Pseudomonas aeruginosa* (isolate), *Pseudomonas aeruginosa* ATCC 10145, *Stapylococcus aureus* (isolate), *Stapylococcus aureus* ATCC 12600, *Escherichia coli* (isolate), *Escherichia coli* ATCC 11775, *Candida albicans* (isolate) and *Candida albicans* ATCC 18804 using agar well diffusion and agar dilution methods was studied after a period of six months under different storage conditions. The antimicrobial activities of extracts stored in closed cupboard were relatively constant when compared with the fresh extracts, except methanol extract, which showed lower Minimum Inhibitory Concentrations against *Candida albicans* after six months. However exposure to light decreased the antimicrobial activities of the extracts with decrease in the diameter of zones of inhibition. The pH of the extract remained stable at 11.0 – 11.5. The implications of these findings in the use of *Alchornea cordifolia* leaf are discussed.

*Keywords*: Stability, *Alchornea cordifolia*, Storage, Antimicrobial activity, Phytochemical screening,

## Introduction

*Alchornea cordifolia* which belongs to the family Euphorbiaceae grows to a considerable height but is always of a shrubby or scrambling habit. It is geographically distributed in secondary forests usually near water, moist or marshy places. It is called by different names in various countries where it is found, for example, it is known as Bulora in Senegal, Mand-Hira in Gambia, Bolontai in Sierra-Leone, Ayraba in Ghana and in Nigeria Isin (Yoruba) and Bambami (Hausa).

The plant has been documented to have medicinal uses for human purposes. In Senegal, the hot water extract of the leaf is taken orally for veneral disease (1). In Nigeria, Ogungbamila and Samuelsson (2) found the hot water extract of the plant effective against gonorrhoea. The ethanol-water (50:50) leaf extract of *A .cordifolia* was found to be active against *Trichophyton rubrum*, the causative agent of ringworm (3). Extracts from leaves of *A. cordifolia* have been reported to inhibit the growth of bacteria such as *Staphylococcus aureus, S. albus, Escherichia coli*, *Salmonella enteritidis* and *Shigella fleneri* ((4, 5).

Quercetin flavonoids (2), alkaloids and tannins (6) and inulin and alchornine (7) have been reported to be present in *A. cordifolia*. Quercetin, a flavonoid antioxidant found in fruits and vegetables (8, 9)

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has been reported to have anti-inflammatory activity (10). Alkaloids rank among the most efficient and therapeutically significant plant substances. Alkaloids have been reported to have anti-inflammatory activity (11) and anti-diabetic activity (12). Akiyama *et al*., (13); Doss *et al*., (14) reported that tannins have potential antibacterial activity.

Stability of an active substance is the capability of that substance in a specific container/closure system to remain within its physical, chemical, microbiological and toxicological specifications (15). Factors such as enzymatic degradation, environmental conditions, air ($O_2$, $CO_2$), light, heat, water (hydrolysis) and duration of time between storage and usage can lead to instability of the antimicrobial activities of some plant metabolites (16).

Decrease in therapeutic activity of the substance can result from the factors mentioned above, therefore there is need to study the effectiveness of therapeutic agents from plants in relation to these factors.

In this study, the phytochemical screening of the leaf extracts and the antimicrobial activity of fresh leaf extracts of *A. cordifolia* was compared with that of extracts stored in cupboard and extracts exposed to sunlight after six months.

**Materials and Method**

*Collection of plant*
The plant was collected from the side of a flowing stream in Idu-Abuja, Nigeria. The collection was made in October 2001. The plant was identified in the herbarium of the National Institute for Pharmaceutical Research and Development, Idu-Abuja, Nigeria. A deposit of the plant specimen with the NIPRD Herbarium number 4334 is in the Institute for future reference.

*Extraction of plant material*
The leaves of *Alchornea cordifolia* (Muell. Arg.) were air dried and then reduced to coarse power using wooden mortar and pestle.
The water extract (WE) was prepared by macerating 300g of the leaf powder in 2 litres of water and left for 24 hours. The extract was filtered and the filtrate was freeze-dried.

Using soxhlet extractor, 1.0 kg of the powdered leaf were extracted successively and exhaustively each time with each of the various solvents starting from the less polar solvent to the more polar solvent. The solvents used were hexane, ethyl acetate and methanol respectively. After each extraction, the extract was concentrated, dried and weighed.

*Phytochemical analysis of the leaf extracts*
The extracts were subjected to phytochemical analysis to detect the presence of the chemical constituents using standard protocol (17, 18).

*Stability Studies of the leaf Extracts*
Methanol, water and ethyl acetate extracts were kept in transparent bottles at room temperature in a laboratory cupboard while another set of the same extracts were kept in transparent bottles on glass window for six months. The stored extracts were then tested for their antimicrobial activity and compared with that of the fresh extracts.

*Determination of some physical changes of the extracts on storage*
The pH of the leaf extracts were taken at different time intervals. The pH meter was zeroed using

standard buffer solution and then the extracts were analyzed. The taste and colour changes were also observed.

*Susceptibility testing*
Overnight broth cultures were diluted with sterile normal saline appropriately using McFarland scale (0.5 McFarland which is about $10^6$ cfu/ml). The molten sterile nutrient agar (20ml) was poured into sterile petri dish and allowed to set. The sterile nutrient agar plates were flooded with 1.0 ml of the standardized inoculum and the excess was drained off. A sterile cork borer (No. 4) was used to bore about six equidistant cups into the agar plate. One drop of the molten agar was used to seal the bottom of the bored hole, so that the extract will not sip beneath the agar. 0.1ml of the different concentrations (2.0mg/ml – 0.0625 mg/ml) of the extracts was added to fill the bored holes.

One hour pre-diffusion time was allowed, after which the plates were incubated at 37°C for 18 hours. The zones of inhibition were then measured in millimeter. Control plates were prepared and incubated appropriately.

*Determination of the Minimum Inhibitory Concentrations (M.I.C.) and Minimum Bactericidal Concentrations (M.B.C) of the Extracts*
Graded concentrations of the leaf extracts ranging from 2.0mg/ml to 0.0625mg/ml were used. These concentrations in sterile molten nutrient agar plates were prepared using double dilution method.

The solidified leaf extract-agar admixture plates were inoculated with 20µl of standardized 18 hour culture test organism. The inocula were allowed to diffuse into the test agar plates for 30minutes.

The test agar plates were then incubated at 37°C for 18 hours and the lowest concentration of the leaf extract in the test agar plates that showed no growth was considered as the M.I.C. of the leaf extract against the test organism. Gentamicin was used as standard antibiotics.

The concentration of the leaf extract in the test agar plates showing no visible growth was inoculated into sterile nutrient agar containing inactivating agents 3% $^{v}/_{v}$ Tween 80 plates. These plates were then incubated at 37°C for 24hours after which they were examined for presence or absence of growth. The plates that yielded less than six colonies were taken as the M.B.C.

**Results**
The result of the phytochemical screening of the *Alchornea cordifolia* leaf extracts revealed the presence of tannins, flavonoids, saponin, alkaloid, glycoside and phenol (Table1).

**Table 1:** Phytochemical screening of the *A. cordifolia* leaf

| Extracts | Chemical constituents | | | | | |
|---|---|---|---|---|---|---|
| | Tannin | Flavonoid | Saponin | Alkanoid | Glycoside | Phenol |
| WE | + | - | + | + | - | + |
| ME | + | - | + | + | - | + |
| EAE | + | + | - | + | + | - |

*Key:* WE = Water Extract; ME = Methanol Extract; EAE = Ethyl acetate Extract; + = Present; - = Absent

The diameter of the zones of inhibition produced by the extracts (WE, ME and EAE) stored at room temperature in the cupboard (EC) were slightly lower than those produced by the fresh extracts (FE). These differences were found to be statistically insignificant at 95% confidence level. There was no

significant difference between the antimicrobial activity of the fresh extract (FE) and the extract stored in the cupboard (EC) at different concentrations at p>0.05 (Tables 2-4).

**Table 2:** The Susceptibility of the Test Organisms to the Fresh and Stored Water Extracts

| TEST ORGANISMS | ZONES OF INHIBITION (MM) | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2.0mg/ml | | | 1.0mg/ml | | | 0.5mg/ml | | | 0.25mg/ml | | | 0.125mg/ml | | | 0.0625mg/ml | | |
| | FE | EC | EL | FE | EC | EL | FE | EC | EL | FE | EC | EL | FE | EC | EL | FE | EC | EL |
| *Ps. aeruginosa* | 26± 0.5 | 25± 0.0 | 16± 0.5 | 23± 0.5 | 23± 0.0 | 15± 0.1 | 20± 0.0 | 21± 0.2 | 12± 0.0 | 17± 0.5 | 16± 0.0 | 00 | 13± 0.0 | 13± 0.1 | 00 | 11 ± 0.0 | 11 ± 0.2 | 00 |
| *Ps. aeruginosa* ATCC 10145 | 15± 0.0 | 15± 0.2 | 11± 0.0 | 11± 0.0 | 12± 0.2 | 10± 0.0 | 00 | 10± 0.1 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 |
| *Staph. aureus* | 26± 0.0 | 24± 0.5 | 18± 0.5 | 24± 0.5 | 22± 0.5 | 17± 0.5 | 22± 0.1 | 20± 0.2 | 15± 0.5 | 18± 0.0 | 15± 0.5 | 12± 0.2 | 12± 0.1 | 10± 0.5 | 00 | 00 | 00 | 00 |
| *Staph. aureus* ATCC12600 | 25± 0.2 | 22± 0.2 | 18± 0.2 | 21± 0.5 | 19± 0.1 | 15± 0.4 | 19± 0.1 | 16± 0.5 | 13± 0.2 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 |
| *E. coli* | 12± 0.5 | 12± 0.1 | 10± 0.0 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 |
| *E.coli* ATCC11775 | 23± 0.0 | 21± 0.0 | 14± 0.1 | 18± 0.0 | 18± 1.1 | 12± 0.0 | 16± 0.5 | 15± 0.1 | 10± 0.0 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 |
| *C. albicans* | 13± 0.1 | 13± 0.0 | 11± 0.0 | 11± 0.2 | 11± 0.5 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 |
| *C. albicans* ATCC 18804 | 16± 0.5 | 15± 0.0 | 12± 0.0 | 14± 0.0 | 14± 0.2 | 10± 0.0 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 |

*Key:* FE = Fresh Extract; EC = Extract Stored in Cupboard; EL = Extract Exposed to Sunlight;
The results are expressed as mean ± standard deviation; FE and EC = P>0.05, FE and EL = P< 0.05

**Table 3:** The Susceptibility of the Test Organisms to the Fresh Methanol Extracts and Methanol Extracts Stored for Six Months

| TEST ORGANISMS | ZONES OF INHIBITION (MM) | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2.0mg/ml | | | 1.0mg/ml | | | 0.5mg/ml | | | 0.25mg/ml | | | 0.125mg/ml | | | 0.0625mg/ml | | |
| | FE | EC | EL | FE | EC | EL | FE | EC | EL | FE | EC | EL | FE | EC | EL | FE | EC | EL |
| *Ps. aeruginosa* | 30 ± 0.0 | 29 ± 0.5 | 20 ± 0.0 | 28 ± 0.5 | 26 ± 0.2 | 19 ± 0.1 | 23 ± 0.5 | 20 ± 0.0 | 18 ± 0.5 | 22 ± 0.5 | 19 ± 0.0 | 12 ± 0.5 | 20 ± 0.1 | 18 ± 0.1 | 00 | 17 ± 0.0 | 16 ± 0.1 | 00 |
| *Ps. aeruginosa* ATCC 10145 | 22 ± 1.1 | 20 ± 0.2 | 16 ± 0.3 | 18 ± 0.1 | 17 ± 0.0 | 13 ± 0.1 | 15 ± 0.1 | 13 ± 0.1 | 11 ± 0.0 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 |
| *Staph. aureus* | 28 ± 0.2 | 25 ± 0.2 | 21 ± 0.2 | 25 ± 0.2 | 23 ± 0.4 | 16 ± 0.0 | 21 ± 0.5 | 19 ± 1.0 | 13 ± 0.2 | 15 ± 1.1 | 13 ± 0.2 | 00 | 13 ± 0.4 | 10 ± 0.2 | 00 | 11 ± 0.2 | 00 | 00 |
| *Staph. aureus* ATCC12600 | 25 ± 0.0 | 23 ± 0.2 | 19 ± 0.0 | 22 ± 0.2 | 20 ± 0.1 | 17 ± 0.0 | 19 ± 0.0 | 18 ± 0.3 | 10 ± 0.0 | 15 ± 0.5 | 13 ± 0.4 | 00 | 00 | 00 | 00 | 00 | 00 | 00 |
| *E. coli* | 15 ± 0.3 | 15 ± 0.0 | 12 ± 0.0 | 12 ± 0.5 | 12 ± 0.5 | 10 ± 0.1 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 |
| *E.coli* ATCC11775 | 27 ± 0.5 | 26 ± 0.1 | 20 ± 0.4 | 21 ± 0.1 | 21 ± 0.0 | 14 ± 0.1 | 15 ± 1.1 | 15 ± 0.0 | 10 ± 0.0 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 |
| *C. albicans* | 12 ± 1.1 | 12 ± 0.2 | 10 ± 0.0 | 11 ± 0.0 | 11 ± 0.3 | 00 | 10 ± 0.1 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 |
| *C. albicans* ATCC 18804 | 20 ± 0.4 | 20 ± 1.0 | 15 ± 0.2 | 17 ± 0.3 | 16 ± 0.2 | 12 ± 0.2 | 15 ± 0.3 | 15 ± 0.1 | 10 ± 0.2 | 12 ± 0.4 | 11 ± 0.0 | 00 | 00 | 00 | 00 | 00 | 00 | 00 |

*Key:* FE = Fresh Extract; EC = Extract Stored in Cupboard; EL = Extract Exposed to Sunlight
The results are expressed as mean ± standard deviation; FE and EC = P>0.05, FE and EL = P< 0.05

**Table 4:** The Susceptibility of the Test Organisms to the Fresh Ethyl Acetate Extracts and Ethyl Acetate Extracts Stored for Six Months

| TEST ORGANISMS | ZONES OF INHIBITION (MM) | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2.0mg/ml | | | 1.0mg/ml | | | 0.5mg/ml | | | 0.25mg/ml | | | 0.125mg/ml | | | 0.0625mg/ml | | |
| | FE | EC | EL | FE | EC | EL | FE | EC | EL | FE | EC | EL | FE | EC | EL | FE | EC | EL |
| *Ps. Aeruginosa* | 30 ± 1.1 | 29 ± 0.0 | 22 ± 0.0 | 29 ± 0.2 | 28 ± 0.1 | 17 ± 0.0 | 22 ± 0.5 | 21 ± 0.0 | 16 ± 0.4 | 18 ± 0.0 | 18 ± 0.2 | 13 ± 0.5 | 12 ± 0.0 | 12 ± 0.5 | 11± 0.2 | 11± 0.0 | 11± 0.0 | 00 |
| *Ps. aeruginosa* ATCC 10145 | 23 ± 0.4 | 21 ± 0.0 | 15 ± 0.4 | 20 ± 0.5 | 20 ± 0.0 | 14 ± 0.5 | 19 ± 0.0 | 18 ± 0.0 | 12 ± 0.2 | 13 ± 0.0 | 13 ± 0.2 | 00 | 00 | 00 | 00 | 00 | 00 | 00 |
| *Staph. aureus* | 35 ± 0.0 | 33 ± 0.2 | 25 ± 0.2 | 30 ± 0.0 | 30 ± 0.1 | 20 ± 0.1 | 25 ± 0.4 | 24 ± 0.0 | 14 ± 0.5 | 18 ± 0.1 | 18 ± 1.1 | 12 ± 0.2 | 15 ± 0.1 | 14 ± 0.2 | 11± 0.0 | 13± 0.0 | 13± 0.0 | 00 |
| *Staph. aureus* ATCC12600 | 26 ± 0.2 | 25 ± 0.0 | 19 ± 0.1 | 23 ± 0.2 | 23 ± 0.0 | 15 ± 0.0 | 19 ± 0.2 | 19 ± 0.4 | 13 ± 0.4 | 16 ± 0.1 | 16 ± 0.2 | 11 ± 0.0 | 10 ± 0.5 | 10 ± 0.3 | 00 | 00 | 00 | 00 |
| *E. coli* | 30 ± 0.2 | 30 ± 0.2 | 21 ± 0.2 | 28 ± 0.2 | 26 ± 0.0 | 18 ± 0.5 | 24 ± 0.0 | 24 ± 0.1 | 16 ± 0.0 | 19 ± 0.2 | 18 ± 0.4 | 13 ± 0.4 | 17 ± 0.1 | 17 ± 0.0 | 10± 0.0 | 12± 0.0 | 12± 0.0 | 00 |
| *E.coli* ATCC11775 | 22 ± 0.0 | 21 ± 0.5 | 15 ± 0.4 | 20 ± 0.5 | 20 ± 0.5 | 13 ± 0.4 | 19 ± 0.2 | 18 ± 0.0 | 11 ± 0.2 | 16 ± 0.1 | 16 ± 0.2 | 00 | 10 ± 0.2 | 10 ± 0.2 | 00 | 00 | 00 | 00 |
| *C. albicans* | 15 ± 0.4 | 14 ± 0.2 | 12 ± 0.4 | 13 ± 0.1 | 13 ± 0.4 | 11 ± 0.1 | 11 ± 0.2 | 11 ± 0.3 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 |
| *C. albicans* ATCC 18804 | 20 ± 0.1 | 20 ± 0.5 | 17 ± 0.2 | 18 ± 0.0 | 17 ± 0.0 | 14 ± 0.2 | 13 ± 0.0 | 12 ± 0.2 | 11 ± 0.1 | 10 ± 0.1 | 10 ± 0.0 | 00 | 00 | 00 | 00 | 00 | 00 | 00 |

*Key:* FE = Fresh Extract; EC = Extract Stored in Cupboard; EL = Extract Exposed to Sunlight;
The results are expressed as mean ± standard deviation; FE and EC = P>0.05, FE and EL = P< 0.05

Generally, the M.I.C. and M.B.C results indicated that the extracts showed a reduced activity after six months i.e. the M.I.C. values increased after six months. However, the activities of the water and methanol extracts against *E. coli* (test and type organisms), water extract against *Ps. aeruginosa* (test organism), and methanol extract against *Ps. aeruginosa* (type organism) remained the same after six months but the activities of water and methanol extracts increased against *C. albicans* (test and type organisms) after the same period (Table 5).

**Table 5:** The M.I.C. and M.B.C of the Fresh Extracts and Extracts Stored for Six Months

| Test Organisms | M.I.C. (mg/ml) (M.B.C [mg/ml]) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Fresh Extracts | | | Exposed to Sunlight for Six months | | | Stored in Cupboard for Six months | | | Gentamicin |
| | WE | ME | EAE | WE | ME | EAE | WE | ME | EAE | |
| *Ps. aeruginosa* | 0.5 (1.0) | 0.5 (1.0) | 0.125 (0.25) | 0.0 | 2.0 (NA) | 2.0 (NA) | 0.5 | 2.0 (NA) | 0.25 | 1.25 |
| *Ps. aeruginosa* ATCC 10145 | 0.5 (1.0) | 0.5 (1.0) | 0.25 | 0.0 | 2.0 (NA) | 2.0 (NA) | 2.0 (NA) | 0.5 | 0.5 | 1.25 |
| *Staph . aureus* | 0.5 (1.0) | 0.125 (0.25) | 0.0625 | 2.0 (NA) | 2.0 (NA) | 1.0 (2.0) | 1.0 (2.0) | 1.0 | 0.25 | 1.25 |
| *Staph . aureus* ATCC 12600 | 0.125 (0.25) | 0.0625 | 0.125 (0.25) | 2.0 (NA) | 2.0 (NA) | 2.0 (NA) | 0.25 | 0.5 | 0.5 | 1.25 |
| *E. coli* | 2.0 (NA) | 2.0 (NA) | 0.125 (0.25) | 0.0 | 0.0 | 0.0 | 2.0 (NA) | 2.0 (NA) | 0.5 | 5.0 |
| *E. coli* ATCC 11775 | 0.5 (1.0) | 0.5 (1.0) | 0.125 (0.25) | 2.0 (NA) | 2.0 (NA) | 2.0 (NA) | 0.5 | 0.5 | 0.5 | 5.0 |
| *C. albicans* | 2.0(NA) | 2.0 (NA) | 0.5 (1.0) | 0.0 | 0.0 | 0.0 | 1.0 (2.0) | 1.0 (2.0) | 1.0 (2.0) | 20.0 |
| *C. albicans* ATCC 18804 | 1.0 (2.0) | 1.0 (2.0) | 0.5 (1.0) | 0.0 | 0.0 | 2.0 (NA) | 0.5 | 0.5 | 1.0 (2.0) | 20.0 |

WE = Water Extract; ME = Methanol Extract; EAE = Ethyl Acetate Extract

The result of the extracts (WE, ME and EAE) exposed to sunlight (EL) indicated that there was drastic reduction in their activities after six months as shown in the diameter of the zones of inhibition when compared with the fresh extracts (Tables 2–4). Generally, at $p<0.05$, there is significant difference between FE and EL inhibitory activities at different concentrations investigated (Tables 2-4). The average difference in the diameters of the zones of inhibition for the extracts were ethyl acetate (7.0 mm), methanol extract (10 mm) and water extract (11.2 mm) (Tables 2-4).

The M.I.C. results indicated that the M.I.C. values of the EL extracts highly increased after six months. The EL extracts completely lost their activities against some of the test organisms. It was generally observed that all the EL extracts (WE, ME, EAE) completely lost their activities against *E. coli* (test organism) and *C. albicans* (test organism). Moreover, the activity of methanol extract was lost against *C. albicans* (type organism); water extract against *Ps. aeruginosa* (test and type organisms) and *C. albicans* (type organism) (Table 5).

**Discussion**

The result of this work revealed the presence of saponins, tannins, glycosides flavonoid, alkaloids, phenols, resins and carbohydrates in the leaf extracts (ME, WE and EAE). These secondary metabolites have been found to be present in plants at various parts and levels of growth. The effectiveness of the fresh leaf juice (19) can be due to the combined action of these secondary metabolites. Previous work on the *Alchornea cordifolia* leaf extracts revealed that tannins isolated from this plant leaf extracts were found to show more antimicrobial activity than the other chemical constituents (20). Several plants which are rich in tannins have been shown to possess antimicrobial activities against a number of microorganisms (14). For example, tannins and alkaloids were detected from *Dichrostachys cinerea* which have antibacterial activities against gram positive bacterial strains more than gram negative bacteria (21) and tannins isolated from *Solarium trilobatum* possessed antibacterial activity (14). It has been reported that alkaloid extracts from *Lupinus angustifolius* (22) and *Genista vuralii* (23) showed significant activity against gram positive bacteria than against gram negative bacterial species. The antimicrobial activity of quercetin flavonoid has been reported against bacterial species (24, 25).

The M.I.C. values of the extracts of the test plant were observed to be significantly compared to the test standard refined antimicrobial agents. These observed activities could be enhanced when the active components of the plants are isolated and examined.

The result of the stability test of the extracts indicated that storage time affected the stability of the extracts. Duration of time between the storage and usage can lead to instability of the active substances (26).

There was no significant difference between the antimicrobial activity of the extracts stored in cupboard and the fresh extracts. Most extracts are believed to be stable at room temperature.

The antimicrobial activities of the extracts exposed to sunlight against the test organisms reduced drastically when compared with the activity of the fresh extracts. It is possible that the antimicrobial activity of the extracts exposed to sunlight reduced due to photodegradation. Natural sunlight lies in the wavelength range 290 – 780 nm of which only the higher energy (U.V.) range (290 – 320 nm) cause photodegradation (27). Many of the chemical constituents found in this plant leaf fall into phenolic group and phenols have been reported to undergo oxidation upon exposure to light (28).

In conclusion, storage time affected the antimicrobial activity of water, methanol and ethyl acetate extracts of *Alchornea cordifolia* leaf. The antimicrobial activities of the extracts were found to reduce with time and in the presence of sunlight over the six months study period, although storage in cupboard at room temperature had no significant effect on the antimicrobial activity of the extracts. To obtain consistent antimicrobial activities in these extracts, fresh ones must be used.

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