AGRICULTURAL AND BIOLOGICAL SCIENCES

ISSN (Print): 24490954
ISSN (Online): 26364972

EVALUATION OF THE EFFICACY OF *ALCHORNEA CORDIFOLIA* MÜLL. ARG., *AGERATUM CONYZOIDES LINN* AND *EUPHORBIA HIRTA LINN* PLANT EXTRACTS AGAINST *VIRULENT ASPERGILLUS* SPECIES

Wuyep P. A., Ezemokwe G.C. and Sila Gyang M.D.

Department of Plant Science and Technology, Faculty of Natural Science, University of Jos, Nigeria

Manuscript Received: 23/07/2017 Accepted: 23/01/2018 Published March 2018

ABSTRACT

Antifungal activities of the aqueous and ethanolic extracts of *Alchonea cordifolia* Müll. Arg., *Ageratum conyzoides Linn*. and *Euphorbia hirta Linn*. plants were evaluated both in vitro and in vivo against *Aspergillus fumigatus, A. niger, A terreus, A, tamarii Kita.* and *A. ustus*; these are mostly implicated in plant and animal fungal diseases. Thin layer chromatography was used to separate and identify the plant components and confirmation of phytochemical screening. Antifungal potential of the extracts were evaluated quantitatively in vitro using well diffusion method and in vivo by studying the survival rate of *Drosophila melanogaster* challenged with the test standard isolates and compared with controls. The percentage yields of aqueous extracts were greater than that of ethanolic. Both extracts showed a potentially good antifungal activity, however aqueous extract had more activity. The activities increased with increasing concentration. Maximum antifungal activity was shown by aqueous of *A. conyzoides* against *A. niger* and *A. ustus* with the average inhibition of 20 mm each while the least activity were extracts of *A. cordifolia* against *A. fumigatus* at the concentration of 800mg/ml with 7mm zones of inhibition. Itraconazole (positive control) at 16.667m/ml, ranged from 15±0.13mm to 20±0.13mm with MIC values from 2.630mg/ml to 6.761 mg/ml. The MIC values of extracts ranged from 50mg/ml to 794mg/ml. The phytochemical screening revealed the presence of some phytochemicals. The activities of the plant extracts against the standard organisms in vivo did not correlate well with the in vitro. The survival behaviours of the infected flies varied. A high survival trend was observed with flies treated with E. hirta followed by *A. cordifolia* while *A. conyzoides* registered high mortality. The extracts showed an antifungal potential both in vitro and in vivo against the standard organisms, confirming the traditional medicinal claims for use against pathogenic fungal infections of plant and animals.

INTRODUCTION

Fungi are ubiquitous in the environment and fungal infections have become more frequent. Fungi are important pathogens of plants and animals with significant yield losses while others spoil crops by producing potent toxins, causing mycotoxicosis in immune-compromised animal and human when infected foods are egested. In addition, some individuals display strong and dangerous allergic reactions to molds (Boundless, 2016). The Food and Agriculture Organization estimates indeed that pests and diseases are responsible for about 25% of crop loss. The genus *Aspergillus* is one of the most commonly implicated (Martínez, 2012).

Control of plant diseases is crucial and has been attempted by use of plants that have been bred for good resistance to many diseases, plant cultivation approaches, recommended fungicide resistance practices, quarantine, and use of pesticide (Fungicides) (Agrios, 1972). The massive and continue use of synthetic fungicides, and the lack of controlled and adequate conditions of usage have generated numerous problems such as new fungal pathogen strains resistant to fungicides and the increase of waste residues and the toxic effects for humans and animals. Fungicide residues in plants tissues following fungicidal treatment, pose a great health risk to the consumer, necessitating a search for safe alternatives to synthetic fungicides. The need to balance these benefits against the risks presents a challenge to the EPA (Environmental Protection Agency) unlike other chemicals. Also, the continuing development of fungicide resistance in plant and human pathogens necessitates the discovery and development of new fungicides (Hrelia *et al.,* 1996).

Continued advances in the science of plant pathology are needed to improve disease control, and to keep up with changes in disease pressure caused by the ongoing evolution and movement of plant pathogens and by changes in agricultural practices (Agrios, 1972). Hence, a wide range of chemicals has been evaluated for their potential for use as alternative to the current fungicides, e.g. plant extracts and some compounds obtained from plants (Wedge and Smith, 2006). Naturally occurring plant products are important sources of antifungal compounds with low toxicity to mammals and safe to the environment which may serve as substitutes for synthetically produced fungicides, as products by themselves or used as starting point for synthesis (Knight *et al.,* 1997). In spite of the previous challenges to the full exploitation of plants, researchers are continually searching new substances naturally occurring in nature with antifungal properties for optimization. Some of these formulates are being already commercialized (Tomlin, 1994).

Since dawn of the civilization, for human and animal treatment, nature has been a treasure of remedies for providing relief from various ailments. Medicinal plants are the richest bio-resource of traditional systems of medicine, modern medicine, nutraceuticals, food supplement, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. Man has continued to search for alternative ways to effective treatment and control, many plants are constantly being screened for their medicinal properties, by carrying out scientific research on them to ascertain, validate and verify their potential, and documented so that acquired knowledge is not completely lost. Phytomedicine have shown great promise in plant including secondary metabolites provide drugs leads for the development of novel therapeutics agents (Pandey *et al.,* 1981).

Literature is flooded with numerous examples of plant derived anti-infection drugs. Among such are *Alchornea cordifolia Müll. Arg*. (*Euphorbiaceae*) commonly called Christmas bush a straggling, laxly branched, evergreen dioecious shrub (Adeshina *et al.,* 2012; Burkill, 1994; Iwu, 1993; Osadebe and Okoye, 2003; Radcliffe-Smith, 1987); *Ageratum conyzoides* Linn. (Asteraceae) commonly called goat weed is a tropical plant (Anjoo and Ajay, 2008; Burkill, 1994; Chah *et al.,* 2006; Fiori *et al.,* 2000) and *Euphorbia hirta* Linn. (Euphorbiaceae), commonly called Asthma plant is a slender- stemmed, annual hairy plant with many branches from the base to top (Burkill, 1994; Evans and Kinghorn, 1975; Galvez *et al.,* 1993; Ma and Tseng, 1997; Sood *et al.,* 2005). Their medicinal as well as other biological properties have been linked to their phytochemicals and metabolites (Abena *et al.,* 1996; Chopra *et al.,* 2002; Horie *et al.,* 1993; Menut *et al.*, 1993; Rastogi and Mehrotra, 2002; Pari *et al.,* 1998; Sharma and Sharma, 1995; Sood *et al.*, 2005; Williamson, 2002; Xuan e*t al.,* 2004; Yamamoto *et al.,* 1991).

Aspergillus spp are one of the most implicated in plant, animal and human fungal diseases. They grow in and on plants and trees, are common contaminants of starchy food and nuts, producing aflatoxin which is both a toxin and carcinogen, and hence the choice of test organisms in this study. Therefore, two extracts (aqueous and ethanolic) of the three plants with medicinal claims which is said to remain poorly studied despite some phytochemical and biological activity studies, would be evaluated by testing its antifungal activities on the standard *Aspergillus* spp standard isolates, in vitro.

MATERIALS AND METHODS

Collection of Plant Materials: The selection of the species used in this study was mainly based on their ethno medical evidence (literature) of use for

conditions related to fungal infections. The leaves of *Alchornea cordifolia* Müll. Arg ; leaves and florescence of A*geratum conyzoides* Linn from and whole plant of Euphorbia hirta Linn were collected from Jos and its environs. Each plant material was labelled, numbered, a noted with the date of collection, locality, and their medicinal uses were recorded.

Identification of Plant Material: The taxonomical identification of the plants was confirmed by plant taxonomists in Department of Plant Science and Technology, university of Jos and deposited at the department's herbarium. Voucher specimen numbers for each plant material were obtained respectively.

Preparation of the Plant Material for Extraction: The plant parts were washed with tap water to remove the adhering dust particles, air dried under shade at room temperature for 14 days and then oven dried for a day to a constant weight and crispy texture to aid grounding to powder using a mortar and pestle and stored in sterile air-tight labeled containers until required (Okigbo and Omodamiro, 2007).

Extraction by Maceration: Ethanolic extraction of plant parts were carried out using modified procedures described by Okigbo and Omodamiro (2007). 400 grams of *A. cordifolia,* 371g of *A. conyzoides* and 305g of E. hirta were soaked in ethanol. The plant powders to ethanol were maintained at the ratio of 1:5 (w/v). The mixtures were kept for 3 days in tightly sealed vessels at room temperature, stirred several times daily with a sterile glass rod. This mixture was filtered through sterile muslin cloth, decanted and filtered using sterile Whatman No. 1 filter paper inserted in a funnel. Batch extraction of the residue was done to increase yield. The filtrates obtained were evaporated to dryness and concentrated under vacuum to dryness under reduced pressure using rotary evaporator at 40^0C to obtain the crude extracts. The same procedure was used for the aqueous extraction 400 grams of *A. cordifolia*, 228 g of *A. conyzoides* and 259g of *E. hirta* plant powder were soaked in distilled water (cold macerated). The ratio of plant to water was maintained at 1:10 (w/v). The filtrates were concentrated by evaporation on water bath at 45 ^{0}C to dryness, not exceeding the boiling point of the solvent (water) (Ezeokeke *et al.,* 2015). The extracts obtained were stored in a refrigerator at 4°C until required for use.

Determination of Percentage Yield: The percentage yield of the crude extract was determined for each solvent (Mahmood 2009; Parekh and Chanda, 2007). The percentage yield of the aqueous and ethanolic extracts can be calculated as thus

$$\textbf{Percentage Yield: } \frac{\text{Weight of plant extract before extraction}}{\text{Weight of plant extract after extraction}} \times 100$$

Purity (Sterility) Test: This is done by streaking a loopful of the extracts on a prepared Potato Dextrose Agar and incubated for appropriate time, approximately 3-7 days at 27°C for possible growth to check for purity and viability (Khan *et al.,* 2006).

Phytochemical Screening: Standard phytochemical test was carried out on the plant samples to determine presence of alkaloids, cardiac glycosides, resin, terpenoids, saponins, tannins, flavonoids, glucosides, and steriods (Sofowora, 1982; Trease and Evans, 1989).

Test Fungi: Standard Isolates of *A. fumigatus, A. niger, A. tamari Kita., A. ustus,* and *A. terreus* was used as previously described by Shugaba *et al.* (2010). The isolates were sub cultured twice on Potato Dextrose Agar PDA from stock before use.

Establishment of Virulence and re-Identification of the Standard Isolates: Stock culture of *A. fumigatus* was streaked onto formulated Yeast Agar Glucose YAG plates and while other *Aspergillus* spp were streaked onto Potato Dextrose Agar PDA, and incubated at 29°C for 3-7 days. Subcultures were produced and colonies identified based on macroscopic colony morphology, micro morphological characteristics, and the ability to grow at 48°C (for A. fumigatus), thereby establishing their virulence (Campbell *et al.,* 1996).

Preparation of Fungal Inoculum: The spores from the surface of the agar plates were collected with inoculating needle and suspended in 3-4 mL of sterile distilled water. The mixture was homogenised and heavy particles were allowed to settle. The homogeneous suspension was adjusted to 0.5 McFarland standards equivalent to the turbidity of the suspension adjusted with a spectrophotometer at 530 nm to obtain a final concentration to match that of a 0.5 McFarland standard for mould (0.4–5 × 106) CFU/ml (CLSI, 2010).

Preparation of Antifungal Stock Solutions: 100g pellet of standard antifungal Itraconazole (of 100% purity) was dissolved in 10ml of 50% acetone sterile distilled water to give a concentration of 10mg/ml, homogenized and centrifuged to separate the binders from the active components, after which 1.2ml of 16.667mg/ml was obtained. A double dilution of this was made (Eloff *et al.,* 2007).

Preparation and Re-constitution of Plant Extracts: For the preparation of dilutions of crude extracts for antifungal assay, the extracts were reconstituted by dissolving in the respective extracting solvents according to modified method described by Elumalai *et al.,* (2009). 4g of the solid plant extract was dissolved in 5mls of 50% acetone in distilled water to make a stock of 800mg/ml and further double dilutions were made to obtain 400mg/ml, 200mg/ml, 100mg/ml, 50mg/ml, 25mg/m, l2.5mg/ml, 6.25mg/ml and 3.125mg/ml. The reconstituted extracts were maintained at a temperature between 2 - 8°C, under

refrigerated condition unless they were used for the experiment (Eloff *et al.,* 2007).

In vitro Antifungal Assay

Antifungal Susceptibility Testing of the *Aspergillus* Isolates to the Plant Extracts: Once the medium had solidified and a sterile 6mm cork-borer was used to bore 9 equidistant wells of 2.5 mm deep on the agar plates. The wells were drilled far from each other to avoid overlap of zone of inhibition, at least 24mm apart, allowing about 10 mm distance to the edge of the plate (Emeruwa 1982; Elumalai *et al.,* 2009). The plant extract 50 µl were placed in wells. The plates were allowed to stand on a level laboratory bench for one hour to allow for proper pre-diffusion of the extract solution into the medium under strict aseptic conditions (Hawaze *et al.,* 2012). The culture was incubated for 48 to 96 hours at 29°C. The evaluation of antimicrobial activity or sensitivities of the microorganism species to the plant extracts were determined by measuring the average sizes of inhibitory zones (including the diameter of wells) on the agar surface around the disks with a meter rule. To account for the inhibitory effect of the solvent, negative and positive controls were included for all pathogens, 50% acetone in sterile distilled water served as negative control while the antifungal drug, Itraconazole served as positive control (Tashiro *et al.,* 2012).

Determination of Minimum Inhibitory Concentration (MIC): A plot of the square of radius diameter of the zones of inhibition against log concentration of the dilutions was done and a suitable curve drawn from the plots of each extracts. Extrapolation of the curves was done to determine the log of MIC. From this log the MIC was calculated as the antilog (Kareem *et al.,* 2012; Otto *et al.,* 2014). The MIC is defined as the lowest concentration that will prevent the growth of the test organisms.

Determination of Minimum Fungicidal Concentration (MFC): The MFC were determined for each of the extracts by sub-culturing the media from each tube or well showing no visible growth in media plates. The plates were incubated at 29°C until growth was seen in the control plates. The MFC is defined as the consequent concentrations required killing 99.9% of the cells (Elumalai *et al.,* 2009; Scorzoni *et al.,* 2007).

Determination of Activity Index (AI): The activity index of the plant material was derived using the formula described by Eloff (2004).

$$\text{Activity index} = \frac{\text{Inhibition Zone of the sample}}{\text{Inhibition Zone of the standard}}$$

Determination of Total Activity (TA): The total activity of the plant material extracted value from one gram of dried plant material was derived using the formula described by Eloff (2004). Total activity (ml/g) =Amount extracted from 1g (mg g-1)/MIC (mg mL-1)

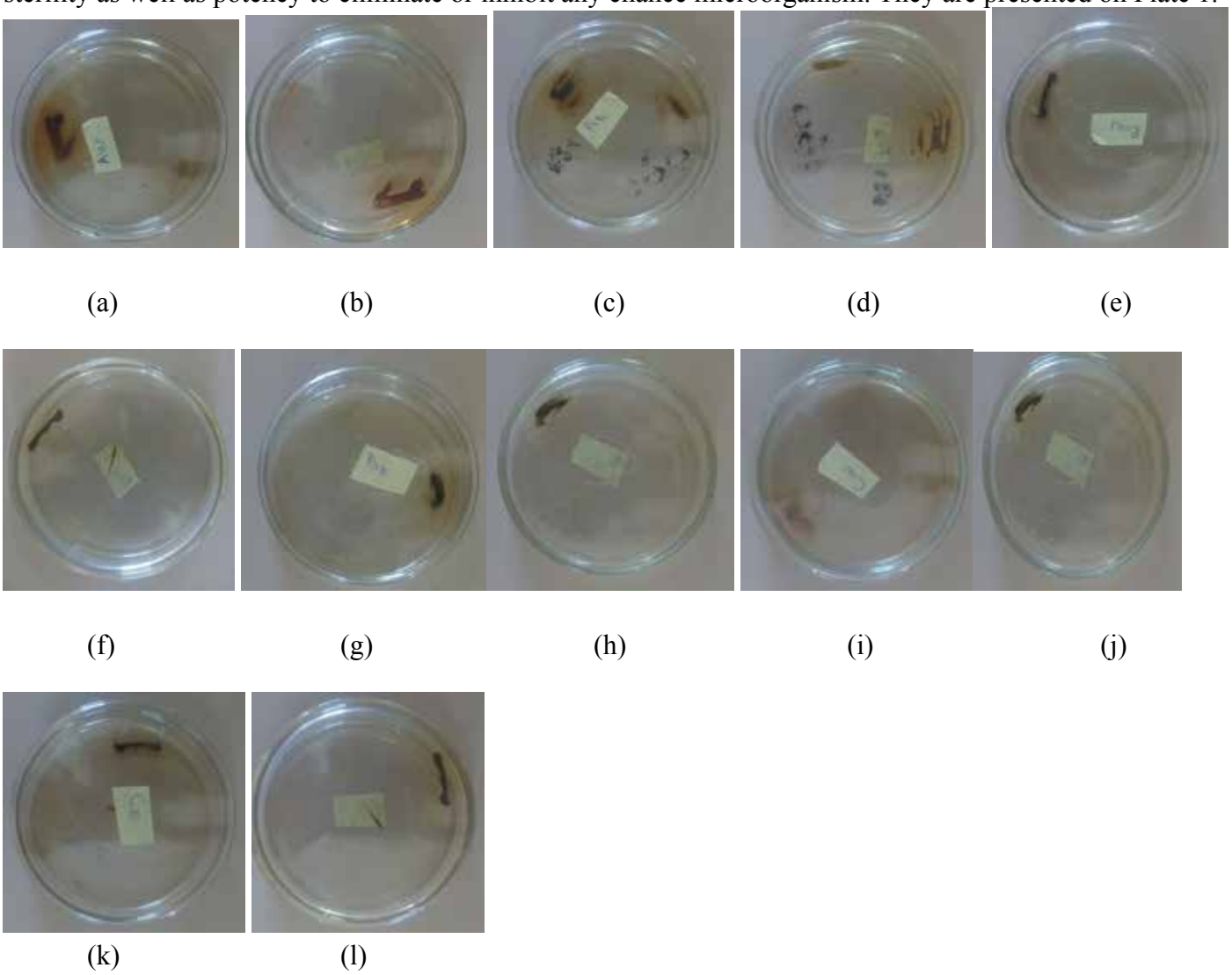
RESULTS AND DISCUSSION

Percentage yields of the aqueous extracts were generally greater than those of ethanolic extracts. The differences in the yields from the two solutions were greatest with *E. hirta* plant. The yield from ethanolic solution was observed to be about 2.5 times greater than that of ethanolic solution. A. cordifolia was the least; yield from aqueous solution was about 1.5 times greater than that of ethanolic solution. The physical characteristics of all the yields from aqueous solution were observed to be brown. Those from ethanolic yields ranged from deep green to brown black. The yields as well as physical characteristics of the crude extract as shown in Table 1.

Table 1: Percentage Yield and Physical Characteristics of the Crude Extract of *Alchornea cordifolia* Müll. Arg., *Ageratum conyzoides* Linn. and *Euphorbia hirta* Linn.

Plant	Extraction Solvent	Raw Plants Powder(g)	Extracted Plants Powder (g)	Percentage Yield (% w/w)	Physical Characteristics
A.cordifolia Müll. Arg.	Aqueous	400	59.775	14.944	Brown
	Ethanolic	400	40.553	10.138	Brown Black
A. conyzoides L.	Aqueous	228	24.615	10 .796	Brown
	Ethanolic	371	23.776	6.409	Black
E. hirta L.	Aqueous	259	58.069	22.420	Brown
	Ethanolic	305	27.243	8.932	Deep Green

Purity (Sterility Test): The percentage purity was determined. It was observed than no growth occurred even up to 10 on Potato Dextrose Agar (PDA) plates streaked with the plant extracts, thereby establishing its sterility as well as potency to eliminate or inhibit any chance microorganism. They are presented on Plate 1.



(a) (b) (c) (d) (e)

(f) (g) (h) (i) (j)

(k) (l)

Keys
A. cordifolia aqueous extract front (a), back (b), ethanolic extract front (c) and back (d)
A. conyzoides aqueous extract front (e), back (f), ethanolic extract front (g) and back (h)
E. hirta aqueous extract front (i), back (j), ethanolic extract front (k) and back (l)

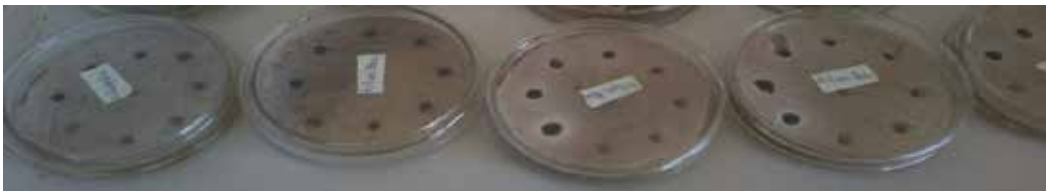
Plate 1: Purity (Sterility) Test for *Alchornea cordifolia* Müll. Arg., *Ageratum conyzoides* Linn. and *Euphorbia hirta* Linn Aqueous and Ethanolic Extracts

The phytochemical investigation of the leaves of *A. cordifolia* Müll. Arg., leaves and florescence of *A. conyzoides L*. and whole plant of *E. Hirta L.* revealed the presence of some phytochemical compounds that are known to exhibit medicinal as well as physiological activities. These were cardiac glycosides, flavonoids phlobatan, steroids, saponins, tannins and terpenods. Cardiac glycoside was observed to present in all three plants while resin was absent. Cardiac glycoside was present in all extracts except aqueous extract of *A. cordifolia*. Alkaloids were absent in all ethanolic extracts but present in all aqueous extracts. Aqueous extracts of *A. cordifolia* and *E. hirta* had the highest metabolites. Aqueous extract had more phytochemicals as well as alkaloids, saponins, steroids and tannins while ethanolic extracts had lesser but revealed the presence of chemical constituents like cardiac glycoside, terpenods, phlobatan, flavonoids and steroids as. There was no result for the confirmation for those of the ethanolic extract on Thin Layer Chromatography, TLC. These results are summarized in Table 2.

Table 2: Phytochemical Screening of Plant Extracts of *Alchornea cordifolia* Müll. Arg., *Ageratum conyzoides* Linn. and *Euphorbia hirta* Linn.

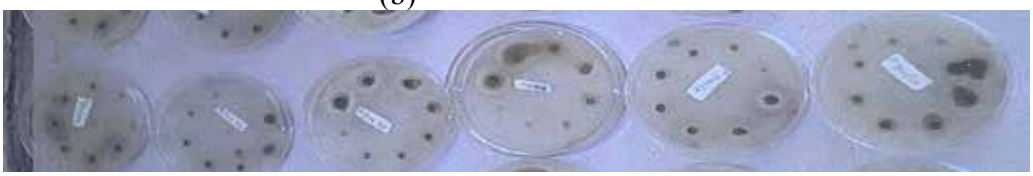
	Extracts					
Phytochemicals	**Aqueous**	**Ethanolic**	**Aqueous**	**Ethanolic**	**Aqueous**	**Ethanolic**
	A. Cordifolia Müll. Arg.		***A. Conyzoides L.***		***E. Hirta L.***	
Alkaloids	+	-	+	-	+	_
Cardiac glycons	+	+	+	+	+	+
Flavonoids	+	+	-	+	+	+
Phlobatan	+	-	-	+	-	+
Resin	-	-	-	-	-	-
Saponins	+	-	+	-	+	-
Steroids	+	-	+	-	+	+
Tannnins	+	-	+	-	+	-
Terpenods	-	+	-	+	+	+

Key
+ = presence
= absence



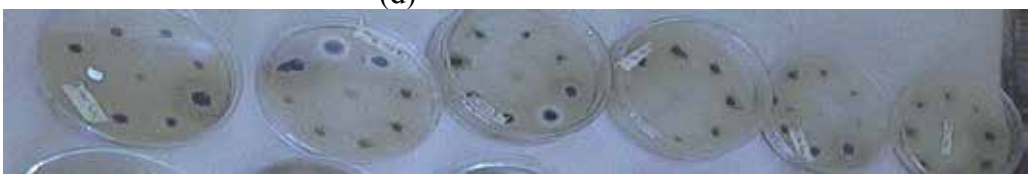
(a)

(b)



(c)

(d)



(e)

Key
(a)=A. fumigatus (b)=A. tamari. Kita (c)=A. niger (d)=A. terreus (e)=A. ustus

Plate 4: Susceptible Testing of aqueus and ethanolic Extracts of *Alchornea cordifolia* Müll. Arg., *Ageratum conyzoides* Linn. and *Euphorbia hirta* Linn against *Aspergillus fumigatus*, *Aspergillus tamari. Kita*, *Aspergillus. niger, Aspergillus terreus* and *Aspergillus ustus*

Antifungal Susceptibility Testing of the *Aspergillus* Isolates to the Plant Extracts and Itraconazole (Positive Control)

The antifungal activity of the plant extracts was assessed by the inhibition of mycelial growth of the *Aspergillus* spp and observed as zones of inhibition near the wells. Zones of the equidistant 9 wells (6mm) containing increasing concentrations from 800mg/ml to 3.125mg/ml (double diluted) was observed to decrease as concentration decreased until there were no zones (Plate 4). Table 3 shows that at concentration of 800mg/ml, A ustus was more susceptible with 12.0±0.9mm followed by A. niger (10±0.2mm), the least were *A. fumigatus* and *A. tamari*. *Kita* but statistically there was no significant difference between them. Aqueous extract inhibited A. ustus up to 200mg/ml. Table 4 shows that at 800mg/ml A. niger was the most effective with 10±0.1mm zone of inhibition followed by A. terreus (8±0.2mm) the rest were 7mm. Non-except that of *A. tamari. Kita* was significantly different. In Table 5, it was observed that the most effective concentration of aqueous extract of *A. conyzoides* was 800mg/ml, A. ustus was most susceptible with 20.0±0.6mm zone of inhibition followed by *A. tamari Kita* (15±0.3) and *A. fumigatus* (8.0±0.1mm) at the least. The zones of inhibition in Table 6 at the highest concentration (800mg/ml) used were statistically not significant, but at 400mg, the differences in zones observed were significant with *A. tamari Kita*. (16±0.3) being most susceptible even up to 50mg/ml. Table 7: At the highest concentration (80mg/ml) used, A. fumigatus was the least susceptible. *A. niger* and *A. tamari Kita* had the highest inhibiting zones 15±0.7mm and 16.0±0.3mm respectively with zones of inhibition up to 50mg/ml. The differences in the aqueous extract of *E. hirta* against the *Aspergillus* spp were not significant at 200, 4000 and 800mg/ml but those of 100mg/ml (A. niger with 7.0±0.3mm zone and *A. tamari. Kita* with 9.0±0.4mm) were significantly different. For Table 8, A. fumigatus was not inhibited at 800mg/ml. The zones of inhibition ranged from 7.0±0.3mm (for A. ustus) to 13.0 ± 0.5mm (for *A. tamari Kita.*) and the difference in their zones were statistically not significant.

Results of antifungal activity are summarized in Table 9. Aqueous and ethanolic extracts of *A. cordifolia, A. conyzoides* and *E. hirta* plants showed a potentially good antimicrobial activity, however aqueous extract showed considerably more activity than the ethanolic extract. Maximum antifungal activity was shown by aqueous of A. conyzoides against A. niger and A. ustus with the average inhibition of 20 mm zone sizes and its ethanolic extract against A. tamari with the inhibition of 16±0.42 mm zone size while the least activity was aqueous and ethanolic extracts of *A. cordifolia* and aqueous extract of *E. hirta* at the concentration of 800mg/ml with 7mm average zones of inhibition, there was no activity for ethanolic extract of *E. hirta*. All the plant extracts showed stronger retardation effect on the *A. niger, A. ustus* and *A. tamarii. Kita*. in descending order while *A. fumigatus* followed by *A. terreus* exhibited less susceptibility. The results obtained also showed that the activities of the extracts increased with increasing concentration. The result showed that the plants extracts significantly ($P<0.05$) differed in their potential to inhibit the growth of the *Aspergillus* isolates.

Antifungal Susceptibility Testing of the *Aspergillus* Isolates to Positive and Negative Controls: Itraconazole, the positive control, inhibited the organisms with values ranging from 20±0.13mm to 15±0.13mm at concentration of 16.667mg/ml. A. terreus was found to be most susceptible while *A. niger* was found to be least susceptible. There was no zone of inhibition observed for the negative controls, distilled water and 50% acetone in distilled water (Plate 6). The same trend was observed at concentration of 8.33mg/ml (Table 4). The differences in the zones observed between the two concentrations were greatest for *A. ustus*.

Table 3: Antimicrobial Activity of Aqueous Extract of *Alchornea cordifolia* Müll. Arg on *Aspergillus fumigatus*, *Aspergillus niger Aspergillus tamari Kita Aspergillus terreus* and *Aspergillus ustus* Isolates

Conc(mg/ml)	*A. fumigatus*	*A. niger*	*A. tamari Kita*	*A. terreus*	*A. ustus*
800	7.0±0.1a	10.0±0.2 a	7.0±0.2a	8.0±0.3a	12.0±0.9a
400	-	-	-	7.0±0.1b	10.0±0.5b
200	-	-	-	-	8.0±0.0c
100	-	-	-	-	-
50	-	-	-	-	-
25	-	-	-	-	-
12.5	-	-	-	-	-
6.25	-	-	-	-	-
3.125	-	-	-	-	-
LSD =	0.14	0.18	0.31	0.47	1.55

Footnote: Numbers tagged with different letter alphabet are significant at P=0.05

Key: NS= No significant difference. Conc= Concentration

Table 4: Antimicrobial Activity of Ethanolic Extract of *Alchornea cordifolia* Müll. Arg on *Aspergillus fumigatus, Aspergillus niger Aspergillus tamari Kita Aspergillus terreus* and *Aspergillus ustus* Isolates

Conc (mg/ml)	*A. fumigatus*	*A. niger*	*A. tamari Kita*	*A. terreus*	*A. ustus*
800	7.0±0.2a	10.0±0.1a	7.0±0.4b	8.0±0.2a	7.0±0.4a
400	-	7.0±0.1b	-	7.0±0.6b	-
200	-	-	-	-	-
100	-	-	-	-	-
50	-	-	-	-	-
25	-	-	-	-	-
12.5	-	-	-	-	-
6.25	-	-	-	-	-
3.125	-	-	-	-	-
LSD =	0.3	0.2	0.6	0.95	.0.6

Footnote: Numbers tagged with different letter alphabet are significant at P=0.05
Key: NS= No significant difference. Conc= Concentration

Table 5: Antimicrobial Activity of Aqueous Extract of *Ageratum conyzoides* Linn. on *Aspergillus fumigatus*, *Aspergillus niger Aspergillus tamari Kita Aspergillus terreus* and *Aspergillus ustus* Isolates

Conc (mg/ml)	*A. fumigatus*	*A. niger*	*A. tamari Kita*	*A. terreus*	*A. ustus*
800	8.0±0.1a	10.0±8.7a	15.0±0.3a	13.0±0.4a	20.0±0.6a
400	7.0±0.3b	15.0 ±0.2a	14.0±0.1ab	12.0±0.6a	15.0±0.3b
200	6.5±0.1c	10.0±0.7a	13.0±0.3b	7.0±0.2b	10.0±0.2c
100	-	9.0±0.3a	9.1±1.2c	-	8.0±0.3d
50	-	8.0±0.5a	-	-	-
25	-	-	-	-	-
12.5	-	-	-	-	-
6.25	-	-	-	-	-
3.125	-	-	-	-	-
LSD	0.49	13.25	1.92	1.12	1.14

Footnote: Numbers tagged with different letter alphabet are significant at P=0.05
Key: NS= No significant difference. Conc= Concentration

Table 6: Antimicrobial Activity of Ethanol Extract of *Ageratum conyzoides* on *Aspergillus fumigatus, Aspergillus niger Aspergillus tamari Kita Aspergillus terreus* and *Aspergillus ustus* Isolates

Conc (mg/ml)	*A. fumigatus*	*A. niger*	*A. tamari Kita*	*A. terreus*	*A. ustus*
800	8.0±0.7a	15.0±0.7a	16.0±0.3a	13.0±0.2a	15.0±0.7a
400	7.0±0.1a	13.0 ±0.4b	15.0±0.2b	9.0±0.3b	10.0±0.1b
200	-	10.0±0.5c	10.0±0.3c	8.0±0.1c	8.0±0.1c
100	-	7.0±0.3d	9.0±0.4d	-	7.0±0.1c
50	-	-	8.0±0.1e	-	-
25	-	-	-	-	-
12.5	-	-	-	-	-
6.25	-	-	-	-	-
3.125	-	-	-	-	-
LSD	1.06	1.49	0.99	0.56	1.11

Footnote: Numbers tagged with different letter alphabet are significant at P=0.05
Key: NS= No significant difference. Conc= Concentration

Table 7: Antimicrobial Activity of Aqueous Extract of *Euphorbia hirta* L on *Aspergillus fumigatus*, *Aspergillus niger Aspergillus tamari Kita Aspergillus terreus* and *Aspergillus ustus* Isolates

Conc (mg/ml)	*A. fumigatus*	*A. niger*	*A. tamari Kita*	*A. terreus*	*A. ustus*
800	7.0±0.1a	15.0±0.7a	15.0±0.3a	10.0±0.3a	13.0±0.3a
400	-	13.0 ±0.25b	13.0±0.25b	8.0±0.2b	8.0±0.1b
200	-	10.0±0.5c	12.0±0.3c	7.0±0.1c	-
100	-	9.0±0.1c	7.0±0.2d	-	-
50	-	7.0 ±0.2d	-	-	-
25	-	-	-	-	-
12.5	-	-	-	-	-
6.25	-	-	-	-	-
3.125	-	-	-	-	-
LSD	0.45	1.38	0.8	0.56	0.47

Footnote: Numbers tagged with different letter alphabet are significant at P=0.05
Key: NS= No significant difference. Conc= Concentration

Table 8: Antimicrobial Activity of Ethanolic Extract of *Euphorbia hirta L* on *Aspergillus fumigatus, Aspergillus niger Aspergillus tamari Kita Aspergillus terreus* and *Aspergillus ustu*s Isolates

Conc (mg/ml)	*A. fumigatus*	*A. niger*	*A. tamari Kita*	*A. terreus*	*A. ustus*
800	-	10.0±0.7a	13.0±0.5a	8.0±0.1a	7.0±0.3a
400	-	7.0 ±0.3b	8.0±0.3b	7.0±0.3b	-
200	-	-	7.0±0.1c	-	-
100	-	-	-	-	-
50	-	-	-	-	-
25	-	-	-	-	-
12.5	-	-	-	-	-
6.25	-	-	-	-	-
3.125	-	-	-	-	-
LSD	NS	1.14	0.89	0.47	0.45

Footnote: Numbers tagged with different letter alphabet are significant at P=0.05
Key: NS= No significant difference. Conc= Concentration

Table 9: Susceptibility of *Aspergillus fumigatus, Aspergillus niger, Aspergillus tamarii. Kita, Aspergillus terreus* and *Aspergillus ustus* to Positive (Itraconazole) and Negative Controls (Distilled Water and 50% Acetone in Distilled Water)

Test	Zones of inhibition (mm)			
Organisms	Concentration of Positive and Negative Controls (mg/ml)			
	Itraconazole		Distilled water	50% acetone
	16.667	8.333		
A.fumigatus	20±0.13	16±0.57	-	-
A.niger	15±0.13	13±0.57	-	-
A.tamarii Kita.	18±0.57	15±0.42	-	-
A.terreus	30±0.28	25±0.14	-	-
A.ustus	20±0.99	13±0.42	-	-

Minimal Inhibition Concentration: MICS were determined from the corresponding concentration-response curves. Table 10 shows the values of the mean radius (R^2) (mm) and corresponding antilog of concentrations used. Figures 1-4 shows the resultant excel plats of logs against R^2 (mm^2) for the crude extracts and Itraconazole. The X-intercepts were gotten from equations of the trend line from excel plots of R^2 against log concentration of crude extracts (leaving out the coordinates with 0 values of R^2) and used to calculate the MIC in the table. The MIC values of the extracts ranged from 794 to 50 mg/ml for the tested Aspergillus spp. The aqueous and ethanolic extracts of *A. cordifolia* plant were less active against most of the tested pathogen with MIC value

of 794mg/ml. The lowest MIC value of 44.668mg/ml was observed for ethanolic extract of A. conyzoides against A. tamari Kita. The highest value ranges was observed with all plant extracts against A. fumigatus. Low values were observed for ethanolic and aqueous extracts of *A. conyzoides* against *A. niger (*63.096mg/ml and 79.433mg/ml respectively) and *A. tamari Kita.* (44.668mg/ml and 50.119mg/ml respectively).

The MIC for the control against the test organisms ranged from 6.761 against *A. ustus* to 2.630mg/ml against *A. terreus*. The least MIC for the extracts was observed to be 11 times less potent than the standard antimicrobial drug (3.890mg/ ml). Table 10 shows R^2 (square of mean diameter radius) and X (intercept = MIC) for Itraconazole.

Minimal Fungicidal Concentration: The MFC values of the extracts ranged from 100mg/ml for most of plant extracts against *A. tamari. Kita.,* to 800 mg/ml for most of plant extracts against *A. fumigatus*. They were observed to have greater values but they followed the same trend with the MIC values. Results for MFC of the crude extracts are presented comparatively with the MIC on Table 17.

Table 10: R^2 (Square of Mean Diameter Radius) and X (Intercepts) Values for Itraconazole Minimal Inhibitory Concentration of Positive Control

Test Organisms	R^2	R^2	X	MIC mg/ml)
A.fumigatus	49	25	0.61	4.074
A.niger	20.25	12.25	0.50	3.162
A.tamarii Kita.	36	20.25	0.59	3.890
A.terrus	144	90.5	0.42	2.630
A.ustus	49	12.25	0.83	6.761
Con (mg/ml)	16.667	8.333		
Log Con	1.222	0.921		

Key: -Log X (intercepts) =MIC mg/ml

Total Activity: The total activity showed that the ethanolic extract of E. hirta against *A. fumigatus* had the lowest value (0.00011ml/g) while the highest was of *E. hirta* against *A. niger* (0.00324ml/g), followed by that of aqueous extract *A. conyzoides* to *A. tamari Kita* (0.00215 ml/g) and then (0.00143ml/g) for ethanolic *A. conyzoides* against same *A. tamari Kita*. A. fumigatus had high values for all plant extracts. The results are summarized in Table 11.

Table 11: Total Activity of the *A. cordifolia* Müll. Arg., Crude Extracts

Plants	**Extract**	**Qty mg/g)**	**Total Activity (ml/g)**				
			A. fumigatus	*A. niger*	*A. tamarii.Kita.*	*A. terreus*	*A. ustus*
A.cordifolia Müll. Arg.	Aqueous	0.149	0.00019	0.00039	0.00019	0.00047	0.00084
	Ethanolic	0.101	0.00013	0.00027	0.00013	0.00047	0.00013

Key:
Qty (mg/g): Quantity of extract mg/g dried plant part
Total activity extract per gram dried plant parts: MIC

The antifungal activities of the three plants extracts agreed with previous works; For *E. hirta*, Jackson *et al.,* 2009 reported activity of the plants against *A. niger, A. fumigatus, A. flavus*, and *Rhizopus oryzae*, including *C. albicans*. For crude extract of *A. conyzoides*, Kishore *et al.,* (1982), studied the antifungal activity against *Cryptomium falcatum* and *Rhizoctonia solani* fungi causing ringworm, *Epidermophyton floccosum*, *Trichophyton mentagrophytes* and *Microsporum gypseum,* the inhibition of the mycelia being 80.28, 78.43 and 68.24%, respectively (Mishra *et al.,* 1991). The low antifungal activity observed in vitro for A. cordifolia had a similar trend with the results obtained by Okeke *et al.* (1999). In his study, three strains, all filamentous fungi, were not susceptible to 40 mg/ml highest concentration tested, a related specie, *A. laxiflora* having similar phytochemical to *A. cordifolia*, exhibited stronger antimicrobial activity, particularly inhibiting the growth of *Alternaria* spp. This could possibly be the storage conditions, moisture content, environmental factors, chemical and biochemical interactions and presence of deteriorating microorganisms (Thakur *et al.*, 2012). However their antibacterial activities of the three plants have been established (Adeshina *et al.,* 2012; Alabashi *et al.,* 1999; Akinyemi *et al.,* 2005; Ebi 2001; Ganesan and Krishnaraju, 1995; Huang *et al.,* 2012; Jenifer *et al.,* 2014; Lamikanra *et al.*, 2006; Natarajan *et al.,* 2005; Chah *et al.,* 2006; Mahmood, 2009; Moody *et al.,* 2004; Pandey *et al.,* 1981; Perumal *et al.*, 1999; Pattnaik *et al.,* 1999; Roland *et al.,* 2007; Sahar 2015; Sharma *et al.,* 1978; Sharma *et al.*, 1979; Sudhakar *et al.,* 2006) as well as the essential oil *A. conyzoides* (Ekundayo *et al.,* 1988; Fiori *et al.,* 2000; Pattnaik *et al.,* 1999, Singh *et al.,* 1986) therefore establishing a broad spectrum activity.

The variation in the antifungal activities of the three plants was probably being that the distribution of

active principles within a plant varied in parts the plant. This could explain why the *A. conyzoides* and *E. hirta* extracts show higher antifungal activities compared to the leaf extract of the *A. cordifolia*. Previous studies with leaves of *A. conyzoides*, (Roland *et al.,* 2007) and comparative study of *E. hirta* parts (Mohammad *et al.,* 2010) confirmed this. Lesser activity had been observed from the leaves of *A. cordifolia* (Ganesan and Krishnaraju, 1995). The antifungal activities can differ depending on the phonological stage of the plant. Leaves of *E. hirta* collected from August to December have been reported to show more significant antimicrobial activities (Suresh *et al.,* 2008).

The higher activities of aqueous extracts of the plants observed in this study agreed with some studies. Aqueous extract of *E. hirta* was more effective in inhibiting bacteria associated with wound Akinrinmade and Oyeleye (2010). The aerial part of E. hirta extracted in nonpolar solvents system exhibited weak inhibitory effect toward test microorganisms (Mahmood and Amey (2007). This might be explained by the ability of water to dissolve different types of compounds; polar and non-polar, simple and complex chemical structures. This however, is in contradiction with results from separate studies. Ethanolic extract of *E. hirta* in Mohamed *et al*. (1996) studies displayed antifungal activity when tested against the plant pathogens *Colletotrichum capsici, Fusarium pallidoroseum, Botryodiplodia theobromae, Alternaria alternata, Penicillium citrinum, Phomopsis caricae-papayae* and *A. niger* as well as for most antibacterial activity against medically important bacterial strains (Banso and Mann, 2006; Boer *et al.,* 2005; Huang *et al.,* 2012; Kumar *et al.,* 2007; Ogbulie *et al.*, 2007; Okwori *et al.,* 2007), justifying the traditional use of alcohol in extracting the leaf components of the medicinal plants for application against pathogens (Pandit and Langfield, 2004). The contradiction over the water and ethanolic extract potency could be attributed to the following; the active principle responsible for the antifungal activity could be identical in structures in both extracts, only that they could be more soluble in water than in ethanol extract. It could also be that that the identical active principles in each extract are different in structure, and the one extracted by water could be more potent compared to those extracted by ethanol, or water compared to the ethanol extract may have more amounts of active principles, or less antagonistic impurities.

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