Journal of Academia and Industrial Research (JAIR)
Volume 6, Issue 3, August 2017

ISSN: 2278-5213

Research Article

Phytochemicals from *Ageratum conyzoides* L. Extracts and their Antifungal Activity against Virulent *Aspergillus* spp.

Ponchang Apollos Wuyep[1], Hannatu Dawa Musa[2], Grace Chiemeka Ezemokwe[1], Davou Dung Nyam[1] and Michael Davou SilaGyang[1]

[1]Department of Plant Science and Technology, Faculty of Natural Sciences, University of Jos, Nigeria;
[2]Department of Botany, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Nigeria
wuyepp@unijos.edu.ng*, zhimak.wuyep@gmail.com, ddavounyam@gmail.com, graceezemokwe@gmail.com; +234 8034503176

Abstract

Antifungal activity of the aqueous and ethanolic extracts of *Ageratum conyzoides* plant were evaluated *in vitro* against *Aspergillus fumigatus*, *A. niger*, *A. terreus*, *A. tamarii* and *A. ustus*; these are mostly implicated in plant and animal fungal diseases. The phytochemical screening revealed the presence of some phytochemicals. Antifungal potential of the extracts were evaluated quantitatively *in vitro* using well diffusion method and challenged with the test standard isolates and compared with controls. The percentage yields of aqueous extracts were greater than that of ethanolic extract. 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 extract of *A. conyzoides* against *A. niger* and *A. ustus* with the average inhibition of 20 mm each while the least activity were recorded against *A. fumigatus* at the concentration of 800 mg/mL with 7 mm zones of inhibition. Itraconazole (positive control) at 16.667 mg/mL, ranged from 15±0.13 mm to 20±0.13 mm with MIC values from 2.630 mg/mL to 6.761 mg/mL. The MIC values of extracts ranged from 50 mg/mL to 794 mg/mL. The activities of the plant extracts against the standard organisms *in vivo* did not correlate well with the *in vitro*. The extracts showed an antifungal potential both *in vitro* against the standard organisms, confirming the traditional medicinal claims for use against pathogenic fungal infections of plant and animals.

Keywords: Phytochemicals, *Ageratum conyzoides*, *Aspergillus* spp., antifungal activity, MIC.

Introduction

Fungi are ubiquitous in the environment and fungal infections have become more frequent. Fungi are important pathogens of plants than animals with significant yield losses while others spoil crops by producing potent toxins, causing mycotoxicosis in immunocompromised animal and human when infected foods are ingested. 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 (Martinez, 2012). Fungal pathogens of plants and animals have multifarious effects. Fungal pathogens possess virulence factors that allow them to cause devastating damage to agriculture; causing significant harvest losses of about 10% of harvest that threaten global food security, disease in domesticated animals and leading to human life threatening mycoses with high mortality rate in immunocompromised persons.

The use of most synthetic fungicides have been restricted because of high acute toxicity, long degradation period, pathogen resistance, bad effect on human health, plant, animal and environment at large. A number of challenges need to be addressed to improve our strategies to control fungal pathogenicity. Consequently, some pest management researchers have focused their efforts on developing alternative inputs to synthetic chemicals for controlling pest and diseases as a biological control. One of the eco-friendly approaches is the use of natural products especially plant derived. Many plants are constantly being screened for their medicinal properties, by carrying out scientific research on them to ascertain, validate and verify their potential. Historically, plant extracts have been used as a safe, effective and natural remedy for ailment and diseases in traditional medicine. They have also played significant role in reducing plant borne pathogens and improvement of crop quality.

*Corresponding author

Traditionally, the screening of bioactive compounds involves, a brute force approach that demands huge investment of significant time and resources to identify a single promising lead compound from chemical libraries consisting of up to several million entities, finding an efficacious drug to bring to market have little or no guarantee. Therefore this study was aimed to evaluate the antifungal efficacy of aqueous and ethanolic extracts of *Ageratum conyzoides* Linn. *in vitro* against virulent *Aspergillus* spp.

Materials and methods

Collection and identification of plant materials: Leaves and florescence of *Ageratum conyzoides* Linn. from Jos South Local Government Area, Plateau State Nigeria were collected. 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; *Ageratum conyzoides* Linn. (UJH16000273) was recorded.

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 d and then oven dried for a day to a constant weight and crispy texture to aid grinding to powder using a mortar and pestle and stored in sterile air-tight labeled containers until required (Okigbo and Omodamiro, 2007).

Ethanolic and aqueous extraction (by Maceration)

Ethanolic extraction: Extraction of plant parts was carried out using modified procedures described by Okigbo and Omodamiro (2007). About 371 g of *Ageratum conyzoides* were soaked in ethanol. The plant powders to ethanol were maintained at the ratio of 1:5 (w/v). The suspensions were kept for 3 d in tightly sealed vessels at room temperature, stirred several times daily with a sterile glass rod. The suspension were first filtered through sterile muslin cloth, decanted and then filtered using sterile Whatman No. 1 filter paper inserted in a funnel. The filtrates were concentrated under vacuum to dryness under reduced pressure using rotary evaporator at 40°C to obtain the crude extracts.

Aqueous extraction: The same procedure described above was used for the aqueous extraction. About 228 g of *A. conyzoides* 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°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.

The dry weight of the plant extracts was obtained by the solvent evaporation and weighted. Some portions were used for phytochemical screening, other parameters, and the rest were used for the susceptibility test.

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

$$\text{Percentage yield} = \frac{\text{Wt. of extract before extraction}}{\text{Wt. of extract after extraction}} \times 100$$

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

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

Determination of pH of plant extracts: Two grams of aqueous and ethanolic extracts were dissolved in 10 mL of distilled water. The electrodes of the calibrated pH meter were buffered and then immersed in the homogenate to obtain the pH values (Nath and Nath, 2015).

Test fungi: Standard Isolates of *Aspergillus fumigatus, A. niger, A. tamarii, A. ustus* and *A. terreus* were 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 PDA, and incubated at 29°C for 4 d. Subcultures were produced and colonies were 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 4 mL of sterile distilled water. The mixture was homogenized and heavy particles were allowed to settle. The homogeneous suspension was adjusted to 0.5 McFarland standards equivalent to the turbidity of the

*Corresponding author

suspension adjusted with a spectrophotometer at 530 nm to obtain a final concentration to match that of a 0.5 McFarland standard for mould ($0.4–5 \times 10^6$) CFU/mL (CLSI, 2010).

Preparation of antifungal stock solutions: About 100 g pellet of standard antifungal Itraconazole (of 100% purity) was dissolved in 10 mL of 50% acetone sterile distilled water to give a concentration of 10 mg/mL, homogenized and centrifuged, after which 1.2 mL of 16.667 mg/mL was obtained. A double dilution of this was made (Eloff *et al.,* 2007).

Preparation and re-constitution of plant extracts: The extracts were reconstituted by dissolving in the respective extracting solvents according to modified method described by Elumalai *et al.* (2009). About 4 g of the solid plant extract was dissolved in 5 mL of 50% acetone in distilled water to make a stock of 800 mg/mL and further double dilutions were made to obtain 400, 200, 100, 50, 25, l2.5, 6.25 and 3.125 mg/mL. The reconstituted extracts were maintained at a temperature between 2-8°C, under refrigerated condition until 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, a sterile 6 mm 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 24 mm apart, allowing about 10 mm distance to the edge of the plate (Emeruwa 1982; Elumalai *et al.,* 2009). The plant extracts (50 µL) were placed in wells. The plates were allowed to stand on a level laboratory bench for 1 h 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 h at 29°C. The evaluation of antimicrobial activity or sensitivities of the microorganism species to the plant extracts was determined by measuring the average sizes of inhibitory zones (including the diameter of wells) on the agar surface around the wells 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 was determined for each of the extracts by sub-culturing the medium 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 concentrations required killing 99.9% of the cells (Scorzoni *et al*., 2007; Elumalai *et al.,* 2009).

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 from one gram of dried plant material was derived using the formula described by Eloff (2004).

$$\text{Total activity (mL/g)} = \frac{\text{Amount extracted from 1g (mg g-1)}}{\text{MIC (mg mL}^{-1}\text{)}}$$

Data collection and statistical analysis: Data obtained were subjected to analysis of variance (ANOVA) using statistical package for social science SPSS to know the significance in the zone of inhibition, effectiveness of each plant extract and the susceptibility of the test organism. Least significant difference of p=0.05 was used to compare means.

Results and discussion

Percentage yield: *Ageratum conyzoides* Linn. gave 24.615 g (10.796% w/w) in aqueous solution out of 228 g of powered plant material while that of ethanolic solution gave 23.776 g (6.409% w/w) out of 371 g powdered plant material (Table 1).

Purity (Sterility test): 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.

Phytochemical screening: Phytochemical investigation of the leaves and florescence of *A. conyzoides* L. revealed the presence of some phytochemical compounds that are known to exhibit medicinal as well as physiological activities.

*Corresponding author

Table 1. Percentage yield and physical characteristics of the crude extract of *Ageratum conyzoides* Linn.

Plant	Extraction solvent	Raw plants powder (g)	Extracted plant powder (g)	Percentage yield (% w/w)	Physical characteristics
A. conyzoides L.	Aqueous	228	24.615	10 .796	Brown
	Ethanolic	371	23.776	6.409	Black

Table 2. Phytochemical screening of plant extracts of *Ageratum conyzoides* Linn.

Phytochemicals	Aqueous	Ethanolic
Alkaloids	+	-
Cardiac glycans	+	+
Flavonoids	-	+
Phlobatan	-	+
Resin	-	-
Saponins	+	-
Steroids	+	-
Tannnins	+	-
Terpenoids	-	+

+ = presence; - = absence.

These were cardiac glycosides, flavonoids phlobatan, steroids, saponins, tannins and terpenoids. Cardiac glycoside was observed to be present in all three plants while resin was absent. Aqueous extract had more phytochemicals namely alkaloids, saponins, steroids and tannins while ethanolic extracts had lesser but revealed the presence of chemical constituents like cardiac glycoside, terpenoids, phlobatan, flavonoids and steroids. These results are summarized in Table 2.

pH determination: The pH values for the plant extract homogenates ranged from 4.10 for ethanolic extract to 5.04 for aqueous extracts of *A. conyzoides*.

Antifungal susceptibility testing of the Aspergillus isolates to the plant extracts: It was observed that the most effective concentration of aqueous extract of *A. conyzoides* was 800 mg/mL, *A. ustus* was most susceptible with 20.0±0.6 mm zone of inhibition followed by *A. tamarii* (15±0.3) and *A. fumigatus* (8.0±0.1 mm) at the least. The zones of inhibition at the highest concentration (800 mg/mL) used were statistically not significant, but at 400 mg/mL, the differences in zones observed were significant with *A. tamarii* (16±0.3) being most susceptible even up to 50 mg/mL (Table 3 and 4) .

Antifungal susceptibility testing of the Aspergillus isolates to positive and negative controls: Itraconazole, the positive control, inhibited the organisms with values ranging from 20±0.13 mm to 15±0.13 mm at concentration of 16.667 µg/mL. *Aspergillus 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.

The same trend was observed at concentration of 8.33 mg/mL (Table 5). The differences in the zones observed between the two concentrations were greatest for *A. ustus*. MICS were determined from the corresponding concentration-response curves. Table 6 shows the values of the mean radius (R^2) (mm) and corresponding antilog of concentrations used.

Table 7 shows the values of X (Intercepts) from the graphs and its antilog values representing the MICs. The MIC values of the extracts ranged from 794 to 50 mg/mL for the tested *Aspergillus* spp. The lowest MIC value of 44.668 mg/mL was observed for ethanolic extract of *A. conyzoides* against *A. tamarii*. 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* (63.096 mg/mL and 79.433 mg/mL, respectively) and *A. tamarii* (44.668 mg/mL and 50.119 mg/mL, respectively). The MIC for the control against the test organisms ranged from 6.761 mg/mL against *A. ustus* to 2.630 mg/mL against *A. terreus*. The least MIC for the extracts was observed to be 11 times less potent than the standard antimicrobial drug (3.890 mg/ mL). Table 8 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 100 mg/mL for most of plant extracts against *A. tamarii*, 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 in Table 9.

*Corresponding author

Table 3. Antimicrobial activity of aqueous extract of *Ageratum conyzoides* Linn. on *Aspergillus* spp.

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

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

Table 4. Antimicrobial activity of ethanolic extract of *Ageratum conyzoides* Linn. on *Aspergillus* spp.

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

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

Table 5. Susceptibility of *Aspergillus* spp. to positive (Itraconazole) and negative controls.

Test organisms	Zones of inhibition (mm)			
	Itraconazole		Distilled water	50% acetone
	16.667 (mg/mL)	8.333 (mg/mL)		
A. fumigatus	20±0.13	16±0.57	-	-
A. niger	15±0.13	13±0.57	-	-
A. tamarii	18±0.57	15±0.42	-	-
A. terreus	30±0.28	25±0.14	-	-
A. ustus	20±0.99	13±0.42	-	-

Table 6. Extracts of *A. conyzoides* showing the different log concentration and their corresponding zone of inhibition.

(Test Organisms)	Zones of Inhibition $(R)^2$													
	Aqueous extract (mg/mL)							Ethanolic extract (mg/mL)						
	80	40	20	10	5.0	2.5	1.25	80	40	20	10	5.0	2.5	1.25
Log conc.	2.90	2.60	2.30	2.00	1.69	1.39	1.09	2.90	2.60	2.30	2.00	1.69	1.39	1.09
A. fumigatus	1	0.25	0.062	0	0	0	0	1	0.25	0	0	0	0	0
A. niger	49	20.25	4	2.25	1	0	0	20.25	12.25	4	0.25	0	0	0
A. tamarii	20.25	16	12.25	4	0	0	0	25	20.25	4	2.25	1	0.25	0
A. terrus	12.25	9	0.25	0	0	0	0	12.25	2.25	1	0	0	0	0
A. ustus	49	20.25	4	1	0	0	0	20.25	4	1	0.25	0	0	0

Where R^2=mean radius (mm)=({mean diameter inclusive of well diameter (mm)}-{well diameter (6 mm)})/2.

Table 7. Extracts of *A. conyzoides* showing MIC and X (Intercepts).

Extract	Test organisms									
	A. fumigatus		*A. niger*		*A. tamarii*		*A. terreus*		*A. ustus*	
	X	MIC	X	MIC	X	MIC	X	MIC	X	MIC
Aqueous	2.30	199.526	1.90	79.433	1.70	50.119	2.25	177.828	2.10	125.893
Ethanolic	2.50	316.228	1.80	63.096	1.65	44.668	2.32	208.930	2.15	141.254

***Corresponding author**

Table 8. MIC of positive control (Itraconazole) showing R^2 (square of mean diameter radius) and X (Intercepts) Values.

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	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
Conc. (mg/mL)	16.667	8.333	-	-
Log Conc.	1.222	0.921	-	-

Log X (intercepts) = MIC mg/mL.

Table 9. Extracts of *A. conyzoides* showing MIC and MFC values.

Extract	Test organisms									
	A. fumigatus		*A. niger*		*A. tamarii*		*A. terreus*		*A. ustus*	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
Aqueous	200	200	79	200	50	100	178	200	126	200
Ethanolic	316	400	63	200	45	100	209	400	141	200

Table 10. Activity index of *Ageratum conyzoides* extracts.

(Test Organisms)	Zones of Inhibition $(R)^2$										
	Aqueous extract (mg/mL)					Ethanolic extract (mg/mL)					
	800	400	200	100	50	800	400	200	100	50	25
A. fumigatus	0.4	0.35	0.325	-	-	0.4	0.35	-	-	-	-
A. niger	1.33	1.00	0.67	0.60	0.53	1.00	0.87	0.67	0.47	-	-
A. tamarii	0.83	0.78	0.72	0.56	-	0.89	0.83	0.56	0.50	0.44	0.39
A. terrus	0.43	0.40	0.23	-	-	0.43	0.30	0.27	-	-	-
A. ustus	1.00	0.75	0.50	0.40	-	0.75	0.50	0.40	0.35	-	-

Activity index: The aqueous extract of *A. conyzoides* against *A. niger* had the highest value of 1.33 at 800 mg/mL, while the least (0.4) was ethanolic and aqueous extracts of *A. conyzoides* against *A. fumigatus* at 800 mg/mL. The values of the activity index are summarized in Table 10.

Total activity: The total activity showed that the aqueous extract of *A. conyzoides* against *A. tamarii was* (0.00215 mL/g) and 0.00143 mL/g for ethanolic *A. conyzoides* against same *A. tamarii*. *Aspergillus fumigatus* had high values for all plant extracts.

Discussion

The phytochemicals revealed in *Ageratum conyzoides* Linn. plant extracts had been documented in previous works (Duke and Ayensu, 1985; Perumal *et al*., 1999; Anjoo and Ajay, 2008; Mahmood 2009; Sandeep *et al.,* 2009; Dressler *et al*., 2014; Ezeokeke *et al*., 2015). That for *A. conyzoides* could be due to the presence of tannins and phenols was probably due to the whole plant part used. Other factors that might have been responsible for the variations are differences in extraction methods, nature of solvent, solvent concentration and polarity, part of plant used as well as the age (Folashade *et al.,* 2012).

These phytochemicals been linked to various activities such as antimicrobial, hepatoprotective, cardioprotective and antioxidant, wound healing, hepatocholesterolemic, spermicidal, insecticidal, anthelminthic, molluscicidal and contraceptive activities and evidential from its traditional uses already mentioned in the literature (Radcliffe-Smith, 1987; Iwu, 1993; Galvez *et al*., 1993; Burkill, 1994; Ganesan and Krishnaraju, 1995; Sharma and Sharma, 1995; Ebi, 2001; Williamson, 2002; Jagetia *et al*., 2003; Tona *et al*., 2004). For example, polyphenolic compounds like flavonoids exhibit various biological activities and are attributed to their ability to form complex with microbial extracellular wall. The antifungal activities of the *A. conyzoides* plant extracts agreed with previous works; 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 higher activity of Itraconazole was expected since the extracts have various impurities as compared to the drug that is already a synthetically processed molecule and has undergone refining processes that have established it as a standard antifungal.

***Corresponding author**

It kills sensitive fungi by interfering with the formation of the fungal cell membrane (Shi *et al.,* 2011). The zones of inhibition of the standards and extracts, though of same volume varied slightly in the replicates, this might be due to uneven distribution of test organisms on agar surfaces or slight difference in temperature and the flatness of the plates at the time it was used. The MIC values found to be lower than the MBC values suggesting that extracts were fungistatic at lower concentrations and fungicidal at higher concentrations. The high MIC and MBC values despite the rich composition of phytochemicals could possibly be attributed to; slight difference in the active component in various parts of the plant, method and solvent used, possible resistant strains used or possible interference with the bioactive components by intrinsic or extrinsic factors. The effectiveness of the plant materials against microorganisms was also based on the activity index and total activity of the plant. It is a measurement of potency of the plant to inhibit the microbial growth. These values indicated the volume that can be added in dried plant material without losing the ability to kill microorganisms (Eloff, 2004).

Conclusion

In conclusion, the aqueous and ethanolic extracts of *Ageratum conyzoides* Linn. inhibited the growth of *Aspergillus fumigatus, A. niger, A. tamari, A. terreus* and *A. ustus* standard isolates *in vitro*. This is an indication that they are potential sources of antifungal compounds and thus supports the uses traditionally for the treatment of various infections and diseases of plant and animals caused by fungal pathogens. Hence, these plants can be used to discover bioactive natural products that may serve as leads. Although, the antifungal properties of the plants seem to have been determined, it is recommended that the guided bioassay for isolation, purification, identification and quantification of the identified bioactive components (bands) are still needed and more studies are also required to reveal the structure activity and relationship of these active constituents. The activity of the extracts against more zygomycetes virulent to plants and animal to be carried out and that the toxicology effects of the plant both *in vitro* and *in vivo* to be carried out to check the environmental safety to plant and animal.

Acknowledgements

Authors are grateful to Rev. Ayewah for helping with the phytochemical extractions and Mrs. Dalen with Fungal Identification.

References

1. Anjoo, K. and Ajay, K.S. 2008. *Ageratum conyzoides* L.: A Review on Its Phytochemical and Pharmacological Profile. *Int. J. Green Pharm.* 2(2): 59-68.
2. Boundless. 2016. Fungi as plant, animal, and human pathogens. *Bound. Biol.*, pp. 600-11819.
3. Burkill, H.M. 1994. The useful plants of West Tropical Africa. Royal Botanical Gardens: Kew.
4. Campbell, C.K., Johnson, E.M., Philpot, C.M. and Warnock, D.W. 1996. Identification of Pathogenic Fungi. London: Public Health Laboratory Service.
5. CLSI, 2010. Method for antifungal disk diffusion susceptibility testing of nondermatophyte filamentous fungi, Approved Guideline, CLSI Document M51-A. Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087, USA.
6. Dressler, S., Schmidt, M. and Zizka, G. 2014. *Alchornea cordifolia*. *African Plants – A Photo Guide*. Frankfurt/Main: Forschungsinstitut Senckenberg.
7. Duke, J.A. and Ayensu, E.S. 1985. Medicinal plants of China, Algonae, Michigan. Reference Publications 2v (Medicinal Plants of the World, No. 4).
8. Ebi, G.C. 2001. Antibacterial activity of *Alcohonea cordifolia* stem Bark. *Fitoterap.* 72(1): 69-72.
9. Eloff, J.N., Picard, L. and Masoko, P. 2007. Resistance of animal fungal pathogens to solvents used in bioassays. *South Afri. J. Bot.* 73: 667-669.
10. Elumalai, E.K., Sivamani, P., Thirumalai, T., Vinothkumar, P., Sivaraj, A. and David, E. 2009. *In vitro* antifungal activities of the aqueous and methanol extract of *Abrus precatorius* Linn (*Fabaceae*) Seeds. *Pharmacol. Online.* 2: 536-543.
11. Emeruwa, A.C. 1982. Antibacterial substance from *Carica papaya* extract. *J. Nat. Prod.* 45: 123-127.
12. Ezeokeke, E.E., Ene, A.C. and Igwe, C.U. 2015. *In vivo* anti-plasmodial effect of ethanol and aqueous extracts of *Alchornea cordifolia*. *Biochem. Analyt. Biochem.* 4: 4.
13. Folashade, K.O., Omoregie, E.H. and Ochogu, A.P. 2012. Standardization of herbal medicines–A Review. *Int. J. Biodiv. Conserv.* 4: 3.
14. Galvez, J., Zarzuelo, A., Crespo, M.E., Lorente, M.D., Ocete, M.A. and Jimenez, J. 1993. Antidiarrheal activity of *Euphorbia hirta* extract and isolation of an active flavonoid constituent. *Planta Medica.* 59: 333-336.
15. Ganesan, T. and Krishnaraju, J. 1995. Antifungal properties of wild plants-II. *Adv. Plant Sci.* 8: 194-196.
16. Hawaze, S., Deti, H. and Suleman, S. 2012. *In vitro* antimicrobial activity and phytochemical screening of Clematis species indigenous to Ethiopia. *Ind. J. Pharmaceut. Sci.* 74(1): 29-35.
17. Iwu, M.M. 1993. Handbook of African medicinal plants. CRC Press, Boca Raton: Florida. pp.464.
18. Jagetia, G.C., Shirwaikar, A., Rao, S.K. and Bhilegaonkar, P.M. 2003. Evaluation of the radioprotective effect of *Ageratum conyzoides* Linn. extract in mice exposed to different doses of gamma radiation. *J. Pharm. Pharmacol.* 55: 1151.
19. Kareem, K.T., Ezeh, A.R., Obi, C.C., Egberongbe, R.K., Jabbar, N.O. and Awoyera, J.O. 2012. *In-vitro* antimicrobial activities of *Euphorbia hirta* against some clinical isolates. *Agricult. Biol. J. North Amer.* 3(4): 169-174.

***Corresponding author**

20. Khan, M.A., Iqbal, Z., Sarwar, M., Nisa, M., Khan, M.S., Lee, W.S., Lee, H.J. and Kim, H.S. 2006. Urea treated corncobs ensiled with or without additives for buffaloes: Ruminal characteristics, digestibility and nitrogen metabolism. *Asian-Australasian J. Anim. Sci.* 19(5): 705-712.
21. Kishore, N., Dubey, N. K., Tripathi, R. D. and Singh, S. K. 1982. Fungi toxic activity of leaves of some higher plants. *Nat. Acad. Sci. Lett.* 5: 9-10.
22. Mahmood, A.M. and Ameh, J.M. 2007. *In-vitro* antibacterial activity of *Parkia biglobosa* (Jacq) root, bark extract against some microorganisms associated with urinary tract infections. *Afri. J. Biotechnol.* 6(11): 195-200.
23. Mahmood, M.A. 2009. Antibacterial activity of crude extracts of *Euphorbia Hirta* against some bacteria associated with enteric infections. *J. Med. Pl. Res.* 3(7): 498-505.
24. Martínez, J.A. 2012. Fungicides for plant and animal diseases. Publisher InTech.
25. Mishra, D.N., Dixit, V. and Mishra, A.K. 1991. Mycotoxic evaluation of some higher plants against ringworm causing fungi. *Ind. Drugs.* 28: 300-303.
26. Nath, S. and Nath, T.K. 2015. Preparation of bio-voltaic cell from the extract of different plant parts. *Int. J. Innov. Res. Rev.* 3(1): 2347-4424.
27. Okigbo, R.N. and Omodamiro, O.D. 2007. Antimicrobial effect of leaf extracts of Pigeon Pea (*Cajanus cajan* (L.) Millsp.) on some human pathogens. *J. Herbs Spices Med. Pl.* 12(1-2): 117-127.
28. Otto, R.B.D., Ameso, S. and Onegi, B. 2014. Assessment of antibacterial activity of crude leaf and root extracts of *Cassia alata* against *Neisseria gonorrhoea*. *Afri. Health Sci.* 14(4): 840-848.
29. Parekh, J. and Chanda, S.V. 2007. *In vitro* antimicrobial activity and phytochemical analysis of some indian medicinal plants. *Turk. J. Biol.* 31: 53-58.
30. Perumal, S.R., Igancimuthu, S. and Patric, R.D. 1999. Preliminary screening of ethnomedicinal plants from India. *J. Ethnopharmacol.* 66: 235-240.
31. Radcliffe-Smith, A. 1987. *Euphorbiaceae* (Part 1). In: Polhill, R.M. (Editor). *Flora of Tropical East Africa.* Rotterdam, Netherlands. pp.407.
32. Sandeep, P., Paarakh, M., and Usha, G. 2009. Antibacterial activity of *Jasminum grandiflorum* Linn leaves. *J. Pharm. Res.* 2(7): 1206-1207.
33. Scorzoni, L., Benaducci, T., Almeida, A.M.F., Silva, D.H.S., Bolzani, V.S. and Gianinni, M.J. 2007. The use of standard methodology for determination of antifungal activity of natural products against medical yeasts *Candida* sp. and *Cryptococcus* sp. *Braz. J. Microbiol.* 38: 391-397.
34. Sharma, P.D. and Sharma, O.P. 1995. Natural products chemistry and biological properties of the Ageratum plant. *Toxicol. Environ. Chem.* 50: 213.
35. Shi, W., Nacev, B.A., Aftab, B.T., Head, S., Rudin, C.M. and Liu, J.O. 2011. Itraconazole side chain analogues: Structure–activity relationship studies for inhibition of endothelial cell proliferation, vascular endothelial growth factor receptor 2 (VEGFR2) glycosylation and hedgehog signaling. *J. Med. Chem.* 54(20): 7363–7374.
36. Shugaba, A., Wuyep, P.A., Nok, A.J., Ameh, D.A. and Lori, J.A. 2010. Bioremediation of hexavalent chromium and tannic acid in synthetic tannery wastewater using free and calcium alginate-immobilized spores and mycelia of *Aspergillus niger* and *Aspergillus parasiticus*. *Bioremed. J.* 14(3): 142-149.
37. Sofowora, E.A. 1982. *Medicinal Plants and Traditional Medicine in Africa*. John Wiley and Sons Limited: Chichester. pp.198.
38. Tashiro, T., Izumikawa, K., Tashiro, M., Takazono, T., Morinaga, Y., Yamamoto, K., Imamura, Y., Miyazaki, T., Seki, M., Kekeya, H., Yamamoto, Y., Yanagihara, K., Yasuoka, A. and Kohno, S. 2011. Diagnostic significance of *Aspergillus* species isolated from respiratory samples in an adult pneumology ward. *Med. Mycol.* 49(6): 581-587.
39. Tona, L., Cimanga, R.K., Mesia, K., Musuamba, C.T., DeBruyne, T., Apers, S., Hernans, N., Van Miert, S., Pieters, L., Totte, J. and Vlietinck, A.J. 2004. *In vitro* antiplasmodial activity of extracts and fractions from seven medicinal plants used in the democratic republic of Congo. *J. Ethnopharmacol.* 93(1): 27-32.
40. Williamson, E.M. 2002. Major Herbs of Ayurveda. China: Churchill Livingstone.

***Corresponding author**