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In Vitro Inhibition of the Vegetative Growth of the Fungus, *Hendersonia celtifolia*, Associated with Foliar Leaf Spots of *Erythrina senegalensis*, Using the Leaf Extracts of *Vernonia amygdalina* L and *Aspilia africana* L

Ibiam OFA[1]* and Nwalobu IP[2]

[1]*Department of Biology/Microbiology/Biotechnology, Federal University, Ndufu-Alike Ikwo, Ebonyi State, Nigeria*
[2]*Department of Biological Sciences, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria*

Abstract

This study is to determine potential use of crude leaf extracts of *Vernonia amygdalina* and *Aspilia africana* for the control of *Hendersonia celtifolia* isolated from leaves of *Erythrina senegalensis* at concentrations: 500 mg/ml, 1000 mg/ml, 2000 mg/ml, 25000 mg/ml. The result of this study showed that there was significant difference (P>0.05) between the treatment and the control. The result also showed that from the first day, the level of growth of the fungus was significantly higher in the control than in the treatment. The effect of the leaf extracts on fungal growth was high as the concentrations increased, and there was a significant variation (P>0.05) in the treatment. The level of inhibition of the vegetative growth of fungus was concentration dependent as increase in concentration of the test leaf extracts from the two plants caused decrease in the vegetative growth of the fungal organism. Comparatively, the anti-fungal activity of leaf extracts from both plants showed that the leaf extracts of *Apilia africana* gave a better inhibitory activity than that of *Vernonia anygdalina* from concentrations 1500 mg/ml to 2500 mg/ml.

Keywords: *In vitro*; Extracts; Management; Leaf spots; Fungus

Introduction

Erythrina senegalensis, is a tree in the pea family that grows in West-African tropical and sub-tropical areas from Senegal to Cameroon. It is a plant that is used in traditional medicine to cure several diseases. This plant is mainly used against amenorrhea, but also against different types of infections [1]. Several traditional uses are still to be explored both *in vitro* and *in vivo* bioassays [2]. A series of Pterocarpans with biological activity have been isolated from this plant, one of which is a new natural product. There are no known threats to this coral tree, which is quite widespread and widely planted, though the extensive use of its bark for medicine often causes trees to be almost stripped of their bark [3]. *Erythrina senegalensis* is planted as an ornamental and used for hedging [4].

Fungal diseases have been recorded on at least 15 *Erythrina* spp. throughout the tropics including leaf spots, mildews, moulds, scorches and blights [5]. Scab caused by *Elsinoe erythrinae* causes defoliation in Brazil and rusts caused by *Dicheirinia binata, Phakopsora pachyrhizi* (soybean rust) and *Uredo erythrinae* are widely reported on *Erythrina* spp. in Mexico, Central and South America, and the Caribbean. Fungal leaf diseases tend to develop circular or halo patterns on the leaves with the dead interior area a tan colour and the surrounding dying tissue a yellow or red color. The affected tissue often has a dry, papery feel [5].

Phytochemical or phytonutrient examination of *Aspilia africana* leaves has shown that the herb encloses active alkaloid, flavonoid, and steroid compounds along with anthraquinones and carbohydrates [6]. The leaves enclosed both aldehydes and ketones and the roots only contain flavonoids [7,8] Misari [8] reported that the root extract also contained plenty of sterols compared to the extracts of the leaves, and the extracts of the roots as well as the leaves of Aspilia were found to have rich contents of alkaloids. While nine active compounds were isolated from the *Aspilia africana* roots, as many as seven compounds were quarantined from the plant's leaves [8]. Tamura et al. [9] reported that methanol extract obtained from *Aspilia africana* possesses anti-microbial actions against *Aspergillus niger*. On the other hand, root extracts of *Aspilia africana* have shown greater effectiveness in inhibiting the growth of *A. niger* [3]. It has been found that compared to the root extracts of Aspilia, the extracts of the plant's leaves have higher capability of inhibiting the growth of microbes, possibly owing to the aldehydes in the roots or further intensive reciprocal or synergic actions of the vigorous compounds occurring in the plant's leaves [2]. All extracts from this herb were found to have actions that inhibit or slow down the growth of microbes.

Anti-nutrient screening of the leaves of *Vernonia amygdalina* revealed the presence of tannins, phlobatannins, flavonoids, steroids, terpenoids, saponins and cardiac glycosides, which are the most important bioactive constituents of medicinal plants. It is reputed to have several health benefits. Saponin content of the leaves have been reported to have antifungal activities [10] and anti-viral activity [11]. The water extract of *V. amygdalina* leaves can inhibit the growth of *Fusarium moniliforme* on seeds of maize (*Zea mays*) as well as mycelial and conidial growths of *Colletotrichum gloeosporioides* in rubber tree [12]. In crop industry, hot water extract of *V. amygdalina* was able to help to control the infection of *Sclerocium rolfii*). Apart from water extract, only methanol extract of *V. amygdalina* was reported to show mild activity on *Rhizoctonia solani* [13]. The ash from burnt branches was used to control seed-borne fungi (*Curvularia*, *Aspergillus*, *Fusarium* and *Penicillium* spp.), thus ameliorating seed viability and germination capacity [14].

Justification for the Work

This coral tree has a large number of traditional medicinal uses in

***Corresponding author:** Ibiam OFA, Department of Biology/Microbiology/Biotechnology, Federal University, Ndufu-Alike Ikwo, Ebonyi State, Nigeria, Tel: +2348035423334; E-mail: drakanuibiamjr@yahoo.com

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West Africa. The bark and roots are used against stomach disorders and as a general tonic, and the bark and leaves are used for dressing wounds. The wood is used for making knife handles, and the seeds are made into necklaces and used as game counters, despite being poisonous. In Ebonyi state, the leaves are eaten with yam, when boiled, and the fresh leaves are added to palm fruits by local farmers to boost extraction of oil from them among other uses for medicinal purposes.

Consequent upon the above and the need to source for alternative to chemical control, the role of higher plants as source of fungicides and their importance in controlling different plant pathogens are gaining prominence in view of the hazards and cost of agro-chemicals. Plant extracts with their biodegradable and eco-friendly nature have shown some promise in recent years. Hence, the decision to use leaf extracts of *Vernonia amygdalina* and *Aspilia africana* in borne out of the need to control fungi that damage our crops and which are also economical compared to the use of synthetic chemicals This study is therefore aimed at *in vitro* determination of the potential inhibitional effect of crude leaf extracts of *Vernonia Amygdalina* and *Aspilia africana* on the vegetative growth of the fungus *Hendersonia celtifolia,* associated with the foliar leaf spot of *Erythrina senegalensis.*

Materials and Methods

Sample collection

Leaves of Aspilia (*Aspilia africana* L) and bitter-leaf (*Vernonia amygdalina* L) were collected from a bush garden farm behind Presco Campus, Ebonyi State University, Abakaliki and was transported to the Department of Applied Biology Laboratory of Ebonyi State University, for identification.

Fugal isolation

20.0 grams of potato dextrose Agar (PDA) was weighed into a 500 ml conical flask and 500 ml of distilled water was added and stirred to dissolve it. The mouth of the flask was sealed with aluminum foil. The medium, along with some 90 cm Oswald Petri dishes wrapped with aluminum foil, were sterilized by autoclaving at 15 psc, at a temperature of 121°C for 15 min. 1 gram of chloramphenicol was added into the medium to inhibit bacterial growth, and stirred, after which it was dispensed immediately into sterile Petri dishes after flaming the mouth of the flask, and allowed to gel. 4 mm pieces of apparently infected parts of the infected *Erythrina senegalensis* were sterilized in 4% sodium hypochcoritee, and rinsed in several changes of sterile distilled water. They were inoculated into the media in the petri dish using sterile forceps (4 pieces) per Petri-dish at equidistant points, and incubated for 48 hours, at 25 ± 2. Pure cultures were made from the fungi isolated. They were identified using their habit character and spore characteristics according to the identification manual by Klich [15] and Barnet and Hunter [3].

Preparation of crude plant extracts

Method according to Nweke and Ibiam [16] modified was used. Fresh mature leaves of the test plant were collected, washed with several changes of sterile distilled water, cut into tiny pieces and air dried to a constant weight for seven days. They were blended with sterile blender and sieved with a fine sterile cheese clothe to obtain some fine powder. Cold aqueous extract of the sample was prepared separately by adding 5 g, 10 g 15 g, 20 g and 25 g of different powdered parts of the test sample into conical flask containing the aqueous solution in the ratio of 1:1, 2:1, 3:1, 4:1 and 5:1 w/v, to give extract concentrations of 500 mg/ml, 1000 mg/ml, 1500 mg/ml, 2000 mg/ml and 2500 mg/ml respectively. They were allowed to mix properly on magnetic stirrer for 5 minutes, and left overnight for extraction of the chemical components of the plant. They were further left in the water bath set at 70°C for 2 hrs for further extraction, and left to cool and stored in the deep freezer until they were needed for the relevant studies.

Test for anti-fungal activities of the leaf extracts

Two mls of the different concentrations of the supernatant of the test extracts were dispersed in 18 ml potato dextrose medium in 9.0 cm Oswald Petri dishes and swirled to blend. 5 mm disc of five days old culture of the test fungus was inoculated at the centre of the PDA medium and incubated at 25°C ± 2°C temperature, and diametric measurement of the growth of the fungus was taken daily for 7 days. The concentrations were made in replicates of three. The control had only the PDA medium without the extract. They were left for extra three days to determine the ability of the fungus to regain vigour and grow against the efficacy of the extracts.

Statistical analysis

All data collected were subjected to statistical analysis by Duncan multiple range test and one way ANOVA using (SPSS 16.0) statistical package and significant differences at p>0.05 using mean and standard deviation.

Results

The result of this study showed that there was significant difference (P>0.05) between the treatment and the control, shown in Tables 1 and 2 for the test samples. The results showed that the level of growth of fungus was significantly higher in the control than in the treatment; the effect of the leaf extracts on fungal growth depending on the concentrations of the extracts of *Vernonia amygdalina* and *Apilia africana.* The growth of the fungus decreased with increase in concentrations of the extracts. Comparing the antifungal activity of both plants, it was observed that the leaf extract of *Apilia africana* showed higher inhibitory activity than leaf extracts of *Venonia anygdalina* (P>0.05). There was a significant variation (P>0.05) between the control and the treatment. The level of inhibition of the fungus was concentration dependent, as increase in concentration led to the inhibition of the vegetative growth of the fungus as show in Tables 1 and 2.

Discussion

The result of this study showed that there was significant difference (P>0.05) between the treatment and the control, as the concentrations of the extracts of *Vernonia amygdalina* and *Aspilia africana* increased. The level of inhibition of fungi was concentration dependent, as increase in concentration led to the inhibition of the growth of fungus as show in Table 2, relatively lesser intensity.

This is similar with the report of Tamura et al. [9], that the methanol extract obtained from *Aspilia africana* had possessed antimicrobial actions against *Aspergillus niger* (*A. niger*). Increased concentration of the extracts led to the decrease in the vegetative growth of the fungus. This effect was significantly decreasing the growth of fungus for six days and from the seventh day, the effect of the extracts on the fungal growth remained constant till the tenth day of the experiments. Ogbebor et al. [12], reported that the water extract of *V. amygdalina* leaves could inhibit the growth of *Fusarium moniliforme* on seeds of maize (*Zea mays*) as well as mycelial and conidial growths of *Colletotrichum gloeosporioides* in rubber tree. In crop industry, hot water extract of *V. amygdalina* was able to help to control the infection of *Sclerotium rolfsii* and increased the plant height, shelf life, relative water content,

Days	Control	500 mg/ml	1000 mg/ml	1500 mg/ml	2000 mg/ml	25000 mg/ml
Day 1	0.21 ± 0.02^{a}	0.15 ± 0.02^{b}	0.12 ± 0.04^{c}	0.12 ± 0.02^{d}	0.11 ± 0.02^{e}	0.11 ± 0.02^{f}
Day 2	0.64 ± 0.12^{a}	0.28 ± 0.01^{c}	0.26 ± 0.01^{b}	0.21 ± 0.02^{d}	0.21 ± 0.02^{e}	0.19 ± 0.01^{f}
Day 3	0.87 ± 0.02^{a}	0.48 ± 0.02^{b}	0.44 ± 0.01^{c}	0.36 ± 0.06^{d}	0.33 ± 0.00^{e}	0.30 ± 0.00^{f}
Day 4	0.96 ± 0.02^{a}	0.53 ± 0.01^{b}	0.53 ± 0.01^{c}	0.47 ± 0.05^{d}	0.42 ± 0.02^{e}	0.40 ± 0.02^{f}
Day 5	1.06 ± 0.02^{a}	0.64 ± 0.01^{b}	0.60 ± 0.06^{c}	0.55 ± 0.075^{d}	0.53 ± 0.02^{e}	0.52 ± 0.02^{f}
Day 6	1.16 ± 0.01^{a}	0.70 ± 0.03^{b}	0.69 ± 0.01^{c}	0.63 ± 0.05^{d}	0.63 ± 0.04^{e}	0.60 ± 0.02^{f}
Day 7	1.27 ± 0.01^{a}	0.83 ± 0.01^{b}	0.77 ± 0.02^{c}	0.75 ± 0.00^{d}	0.71 ± 0.03^{e}	0.71 ± 0.03^{e}
Day 8	1.41 ± 0.03^{a}	0.83 ± 0.01^{b}	0.77 ± 0.02^{c}	0.750 ± 0.00^{d}	0.71 ± 0.03^{e}	0.71 ± 0.03^{e}
Day 9	1.45 ± 0.05^{a}	0.83 ± 0.01^{b}	0.77 ± 0.02^{c}	0.750 ± 0.00^{d}	0.71 ± 0.03^{e}	0.71 ± 0.03^{e}
Day 10	1.45 ± 0.05^{a}	0.83 ± 0.01^{b}	0.77 ± 0.02^{c}	0.750 ± 0.00^{d}	0.71 ± 0.03^{e}	0.71 ± 0.03^{e}
***Means with the same letter are not significantly different (p<0.05) ***Mean with the different letter are significantly different (p>0.05)						

Table 1: Effect of leaf extract of *Vernonia amygdalina* on the vegetative growth of *Hendersonia celtifolia* isolated from *Erythrina senegalensis* leaves.

Days	Control	500 mg/ml	1000 mg/ml	1500 mg/ml	2000 mg/ml	25000 mg/ml
Day 1	0.19 ± 0.01^{c}	0.18 ± 0.00^{d}	17 ± 0.02^{a}	0.16 ± 0.02^{b}	0.14 ± 0.01^{e}	0.13 ± 0.02^{f}
Day 2	0.62 ± 0.06^{a}	0.31 ± 0.02^{b}	0.29 ± 0.04^{c}	0.25 ± 0.02^{d}	0.23 ± 0.01^{e}	0.21 ± 0.01^{f}
Day 3	0.82 ± 0.02^{a}	0.42 ± 0.04^{b}	0.39 ± 0.04^{c}	0.35 ± 0.02^{d}	0.32 ± 0.02^{e}	0.29 ± 0.01^{f}
Day 4	0.89 ± 0.08^{a}	0.52 ± 0.04^{b}	0.49 ± 0.04^{c}	0.47 ± 0.03^{d}	0.43 ± 0.02^{e}	0.41 ± 0.02^{f}
Day 5	1.05 ± 0.02^{a}	0.62 ± 0.06^{b}	0.59 ± 0.06^{c}	0.57 ± 0.06^{d}	0.53 ± 0.05^{e}	0.52 ± 0.02^{f}
Day 6	1.17 ± 0.03^{a}	0.76 ± 0.01^{b}	0.70 ± 0.02^{c}	0.65 ± 0.00^{d}	0.63 ± 0.00^{e}	0.59 ± 0.01^{f}
Day 7	1.27 ± 0.03^{a}	0.85 ± 0.02^{b}	0.74 ± 0.01^{c}	0.70 ± 0.02^{d}	0.66 ± 0.01^{e}	0.63 ± 0.01^{f}
Day 8	1.27 ± 0.03^{a}	0.85 ± 0.02^{b}	0.74 ± 0.01^{c}	0.70 ± 0.02^{d}	0.66 ± 0.01^{e}	0.63 ± 0.01^{f}
Day 9	1.27 ± 0.03^{a}	0.85 ± 0.02^{b}	0.74 ± 0.01^{c}	0.70 ± 0.02^{d}	0.66 ± 0.01^{e}	0.63 ± 0.01^{f}
Day 10	1.27 ± 0.03^{a}	0.85 ± 0.02^{b}	0.74 ± 0.01^{c}	0.70 ± 0.02^{d}	0.66 ± 0.01^{e}	0.63 ± 0.01^{f}
***Means with the same letter are not significant difference (p<0.05) ***Mean with the different letter are significant difference (p>0.05)						

Table 2: Effect of leaf extract of *Aspilia africana* on the vegetative growth of *Hendersonia celtifolia* isolated from *Erythrina senegalensis* leaves.

chlorophyll content, leaf area index, number of branches, total dry matter, number of pod per plant, weight and also grain yield on cowpea [14]. Ohigashi et al. [13], reported that the methanol extract of *V. amygdalina* showed mild activity on *Rhizoctonia solani.*

Comparing the antifungal activity of both plants, it was observed that the leaf extract of *Aspilia africana* showed higher inhibitiory activity than leaf extracts of *Vernonia amygdalina* as shown in Tables 1 and 2.

Conclusion

The result of this study showed that leaf extracts of *Vernonia amygdalina* and *Aspilia africana* inhibited the growth of the fungus. Although, the leaf extract of *Aspilia africana,* showed higher rate of inhibitional potential than leaf extract of *Vernonia amygdalina* and increase in concentration decreased the vegetative growth of the fungi to seventh day, after which it remained constant, the constant growth of the fungus was indicative of the fact that the effect of the extracts of both plants controlled the growth of the fungus.

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