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# ANTIFUNGAL ACTIVITY OF AQUEOUS STEM BARK EXTRACT OF *Pterocarpus erinaceous* and *Erythrophelum suaveolens* (Guill &Perri) Brenan ON SWEET POTATO FUNGI

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***ABSTRACT***

***Sweet potato is an important source of food and income for communities in Benue State North Central Nigeria. The plant is prone to attack by leaf spot fungi which are capable of partially or totally killing the plant. The antifungal activity of three concentrations of aqueous extract of Erythropleum suaveolens and Pterocarpus erinaceous were used in the management of sweet potato leaf spot fungi viz: Macrophomina phaseolina (Tassi) Goid, Aspergillus flavus and Fusarium verticolliodes. Three concentrations of the stem bark extracts of Erythrophelum suaveolens and Pterocarpus erinaceous at 10, 20 and 30% w/v were used in the in-vitro management of leaf spot pathogen of sweet potato. The experiment was a 2×3 factorial laid out in a Completely Randomised Design (CRD). The hot water aqueous extracts of the stem bark of E. suaveolens exhibited fungitoxicity against all three test fungi at 3 and 7 Days After Inoculation (DAI). The mycelia growth of test fungi after three days was significantly lower in Potato Dextrose Agar (PDA) amended with the aqueous extracts of E. suaveolens compared with the aqueous extracts of P. erinaceous. Generally, the results indicated that the test fungi were most sensitive to P. erinaceous at 20% w/v while E. suaveolens recorded the least potency at 20% w/v. The efficacy of stem bark extracts of P. Erinaceous at 10% w/v increased at the seventh day thereby reducing mycelia growth of A. flavus but stimulated the mycelia growth of M. phaseolina. The study demonstrated fungi toxicity of the aqueous crude extract of the stem bark of E. suaveolens against sweet potato fungi.***

**Keywords:** Plant extract, fungi, sweet potato, fungitoxicity, leaf spot.

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## INTRODUCTION

Sweet potato (*Ipomoea batatas*(L) Lam) is a dicotyledenousplant belonging to the morning glory family (Convolvulaceae) whichcontains about 45 genera and 1,000 species of which only *I. batatas* is of economic importance as food (Udemezue, 2019).Sweet potato is the third most important tuber crop after cassava and yamwithin Nigeria and fourth globally (Wariboko and Ogidi, 2014).It is a source of carbohydrate,protein, fats, vitamins and minerals among resource poorfarmers in Southern Guinea Savannah agro - ecology of Nigeria (Egbe, 2012).Its production in Nigeria and Benue State in particular is a competitive and profitable enterprise supporting the rural farmers (Ali *et al*., 2017).

Sweet potato is utilized in various ways. The root can be boiled and combined with cowpea, rice, millet, Benniseed and eaten as food. It can also be roasted or fried into chips and eaten with stew or palm oil (HKI, 2013). The young leaves and shoots are eaten fresh as vegetables in soups and stews or stir fried with chili and dried shrimps

(Tewe *et al.*, 2003). Sweet potato rootsarealso sun-dried, milled into flourand then used in the preparation of bread and cakes or processing into 'fufu' (Adeyonu *et al*., 2016).

The root of sweet potato has a low glycemic index making it a suitable root crop that can be safely consumed by persons with diabetes (Wariboko and Ogidi, 2014). The orange fleshed variety is added as fortification of food fed to young children in order to reduce Vitamin A deficiency in developing countries like Nigeria (Ezeano, 2010).Industrially sweet potato starch is used as adhesive by pharmaceutical, textile, wood and paper industries (Nmorand Okobia, 2017).It is also used in the production of noodles, candy, desserts and flour(Egbe 2012; Wariboko and Ogidi, 2014).The dried sweet potato chips are used for preparing a fermented gruel called 'kunu' in Hausa which is a common drink in Northern Nigeria (Amienyo and Ataga, 2007; Ezeano, 2010). Sweet potato is used in preparation of culture media for fungi and as a composite replacement of wheat in confectionary products and snacks like chin- chin, buns, doughnut and bread (Ezeano, 2010).

The yield of sweet potato in Nigeria is reduced by the high incidences of pests and diseases (Echerenwa and Unechuruba, 2004;Wariboko and Ogidi, 2014).Leaf spot pathogens produce spots on the leaves which reduce the photosynthetic area of the plants and consequently, reduce the amount of assimilate that goes into the photosynthetic sink. Leaf spot disease account for about 20% to 40% losses and are capable of partially or totally killing the plant resulting in crop failure (Boa, 2014). Ekhuemelo and Nsobundu (2020) reported the pathogenicity of *Fusarium verticillioides*, *Aspergillus flavus*, *Aspergillus tamarii and Macrophomina phaseolina* on sweet potato grown in Makurdi, Nigeria resulting in leaf defoliation and reduction of the leaf area.

The stem bark extracts of *E. suaveolens*are used as anesthetic anddisinfectant. It is also used in the treatment of malaria and skin diseases (Akinpelu *et al*., 2012). Recent studies by Ekhuemelo *et al*. (2019b) and Ekhuemelo *et al*.(2019c) reported the antibacterial and antifungal activities of the fractions of hexane and ethyl acetate extracts of *Erythropleum suaveolens* and *Pterocarpus erinaceous* against some plant and human fungi.

The processing of *Erythropleum suaveolens* and *Pterocarpus erinaceous* for woods results in the removal of the stem bark which contribute to wood waste in the environment with the attendant pollution. Previous studies have been focused on the antifungal and antibacterial activities of solvent extracts of *E. suaveolens* and *P. erinaceous* on woodfungi (Ekhuemelo *et al*. 2019a; Ekhuemelo *et al*. 2019b; Ekhuemelo *et al*. 2019c and Ekhuemelo *et al*. 2019d). Aqueous extraction is obtained by infusion process which is cheaper and less cumbersome than the solvent extraction process There is the need to use the stembark which is removed during wood processing of *E. suaveolens* and *P. erinaceous* by utilizing it as botanicals in the management of leaf spot disease of sweet potato. This will reduce leaf spot infection and increase the productivity of sweet potato in the study area.The study was therefore conducted to determine the antifungal effect of three concentrations of the aqueous extract of *Erythropleum suaveolens* and *Pterocarpus erinaceous* in the management of leaf spot pathogens of sweet potato in Makurdi.

## MATERIALS AND METHODS

### Source of Experimental Materials

Infected sweet potato leaves of TIS 8164 showing characteristic leaf spot from a sweet potato plot at the National Root Crop Research Institute Otobi, sub- station (7º 07' - 7º 11'N and 8º 05 - 8º 10'E) were collected and packaged in sterilized paper bags. The samples were transported to the pathology laboratory of the Federal University of Agriculture, Makurdi, Nigeria (Coordinates: Latitude $7.41^0$ N, Longitude $8.35^0$ E) for fungi isolation and management trials.

### Plant sample collection and extraction

The stem bark samples of *Erythrophelum suaveolens* and *Pterocarpus erinaceous* were collected in October, 2018 from the Premises of the Federal University of Agriculture, Makurdi and from around Makurdi City. The dried samples were ground into powder for use in the experiment. Samples corresponding to 10%, 20% and 30% w/v of *Erythrophelum suaveolens* and

*Pterocarpus erinaceous* were heated for 30 minutes, cooled and filtered using double layer cheese cloth.

**Experimental Design and Treatment**

The study on the *in-vitro*management of leaf spot pathogen of sweet potato was a 2x4 experiment consisting of the stem bark extracts of *Erythrophelum suaveolens* and *Pterocarpus erinaceous* at three concentrations of 10%, 20% and 30% w/v and an untreated control. The experiment was laid out in Completely Randomized Design (CRD) and replicated three times.

**Isolation and Identification of Leaf Spot Pathogen**

Two millimeters long section of the infected sweet potato leaves were cut out from the margins of necrotic leaf spot and sterilized for 1 minute in 10% commercial sodium hypochlorite solution after which they were rinsed in three changes of sterile distilled water (SDW) and blotted dry on sterile filter papers. The sweet potato leaves were plated on Potato Dextrose Agar (PDA). The plates were then incubated at ambient conditions of light and temperature (30±2ºC) for 7days. Pare culture was obtained by sub culturing into fresh PDA plates. Microscopic examination was done by examining the colony characteristics; a sterile needle was used in taking a tiny portion of the hyphae containing spores on the sterile glass slides stained with lactophenol cotton blue and examined under the microscope for fungal structures. Pure cultures were identified using compound microscope and compared with reference manual (Watanabe, 2010).

**Fungitoxic Effect of Plant Extracts**

Potato Dextrose Agar (PDA) was added to each filtrate and autoclaved at 121°C for 15minutes. Streptomycin sulphate was added at the rate of 100mg/litre when the media cooled to about 40ºC to prevent bacteria contamination. The media amended with plant extracts were inoculated at the centre with 2mm diameter mycelia discs taken from the advancing edges of 7 days-old pure culture of Macrophomina *phaseolina*, *Fusarium verticolloides* and *Aspergillus flavus*. The inoculated media were incubated at ambient conditions of light and temperature (30±2ºC). The diameter of the fungal colony was measured using a meter rule along two diagonal lines drawn on the reverse side of each Petri dish from 3 days after incubation.

**Data Collection**

Data on mycelia growth was recorded at 3 days and 7 days after inoculation (DAI). The percentage reduction or stimulation of mycelia growth was computed with slight modification of the formula of Nduagu *et al.,* (2008).

$$Mi. = M1 - M2/M1\ x\ 100 \text{---------- (1)}$$

Where
Mi- % inhibition of mycelia growth
$M_1$-mycelia growth on untreated medium (control);
$M_2$- mycelia growth on the treated medium.

$$Ms = M2 - M1/\ M1\ x\ 100 \text{ ----- (2)}$$

Where:
Ms - % stimulation ofmycelia growth
$M_2$- mycelia growth on treated medium;
$M_1$- mycelia growth in untreated medium (Nduagu *et al*., 2008).

The effectiveness of the plant extract was rated as ≤ 0 % inhibition (Not effective), > 0- 20 % inhibition (Slightly effective), > 20-50 % inhibition (Moderately effective), > 50- < 100 % inhibition (Effective), 100 % inhibition (Highly effective) (Ekhuemelo and Yaaju, 2017).

**Data analysis**

Data were subjected to analysis of variance (ANOVA) using SAS version 9.2 statistical software package (SAS, 2009) and significantly different treatment means compared with Fishers Least Significant Difference (FSLD) at 5% level of probability (Obi, 2002).

**RESULTS**

The effect of three concentrations of the aqueous extract of *Erythrophleum suaveolens* and *Pterocarpus erinaceous* on the management of fungi infecting sweet potato three days after inoculation is presented in Table 1. The mycelia growth of test fungi was significantly lower in plates amended with the stembark extract of *E.*

s*uaveolens* compared with the stem bark extracts of *P. erinaceous* three days after inoculation.

The application of *E.* s*uaveolens* at 10 %, 20 % and 30 % w/v significantly ($P \leq 0.05$) reduced the mycelia growth of *Fusarium verticolloides* by 56.50% (effective), 61.46 % (effective) and 64.86% (effective) respectively. The mycelia growth of *Aspergillus flavus* was significantly ($P \leq 0.05$) reduced by *E.* s*uaveolens* at 10 % w/v by 22.98% (moderately effective), 20 % w/v by 41.06 % (moderately effective) and 30 % w/v by 61.28 % (effective). The management of *M. phaseolina* by 10 %, 20 % and 30 % w/v of the aqueous stem bark extract of *E.* s*uaveolens* elucidated mycelia growth inhibition of 46.95% (moderately effective), 60.96 % (effective) and 63.92% (effective).

The stem bark extract of *P. erinaceous* at the three concentrations stimulated the growth of *A. flavus* by 6.56% (slightly effective), 18.26% (slightly effective) and 23.20% (moderately effective) while the stem bark extract of *P. erinaceous* at 10%, 20% and 30%w/v significantly ($P \leq 0.05$) reduced the mycelia growth of *Fusarium verticolloides* by 9.75% (slightly effective), 24.46% (moderately effective) and 40.71% (moderately effective) respectively. The mycelia growth of *M. phaseolina* was reduced by 35.42% (moderately effective) when *P. erinaceous* was applied at 30%w/v.

**Table 1: Effect of three concentrations of the stem bark extract of *Erythrophleum suaveolens* and *Pterocarpus erinaceous* on the control of Sweet Potato fungi three days after inoculation**

| Plant Extract | Concentration of extracts | Mycelia growth | | | Plant extract concentration mean |
|---|---|---|---|---|---|
| | | ***M. phaseolina*** | ***A. flavus*** | ***F. verticollioides*** | |
| ***Erythrophleum suaveolens*** | 10 | 3.22(46.95R) | 3.62(22.98R) | 2.81(56.50R) | 3.21(44.17R) |
| | 20 | 2.37(60.96R) | 2.77(41.06R) | 2.49(61.46R) | 2.54(55.83R) |
| | 30 | 2.19(63.92R) | 1.83(61.28R) | 2.27(64.86R) | 2.10(63.48R) |
| ***Pterocarpus erinaceous*** | 10 | 6.52(6.90S) | 5.75(18.26S) | 5.83(9.75R) | 5.87(2.04S) |
| | 20 | 6.02(No effect) | 6.12(23.20S) | 4.88(24.46R) | 5.84(1.54S) |
| | 30 | 3.92(35.42R) | 5.03(6.56S) | 3.83(40.71R) | 4.26(25.91R) |
| | **Control** | **6.07** | **4.70** | **6.46** | **5.75** |

*FLSD Plant extract = 0.73; Mycelia growth =NS; Plant extract x Mycelia growth = 1.27*
*Values in parenthesis represent percentage (%) Reduction (R) or Stimulation (S) of mycelia growth*.

Data presented in Table 2 shows the effect of three concentrations of the stem bark extract of *Erythrophleum suaveolens* and *Pterocarpus erinaceous* on the management of sweet potato fungi seven days after inoculation. At 7 days after inoculation, *E. suaveolens* at 30 % w/v concentrations significantly reduced the mycelia growth of *M. phaseolina* by 36.83% (moderately effective) while mycelia growth of *M. phaseolina* was reduced by 10% w/v and 20% w/v of stem bark extract of *E. suaveolens* were reduced by 17.79% (slightly effective) and 25.35% (moderately effective) respectively. The mycelia growth of *A. flavus* was significantly reduced by 10%, 20 %, 30% w/v concentrations of *E. suaveolens* tested by 14.91% (slightly effective), 42.36% (moderately effective) and 65.27% (effective) respectively. *Fusarium verticolliodes* growth was reduced by 10%, 20 %, 30% w/v concentrations of the aqueous stem bark extract of E. *suaveolens* by 17.66% (slightly effective), 57.31% (effective) and 20.84% (moderately effective) respectively. *Pterocarpus erinaceous* reduced mycelia growth of *F. verticolloides* by 8.51% (slightly effective), 8.01% (slightly effective) and 13.98% (slightly effective) respectively.

**Table 2: Effect of three concentrations of the stem bark extract of *Erythrophleum suaveolens* and *Pterocarpus erinaceous* on the control of Sweet potato fungi seven days after inoculation**

| Plant Extract | Concentration of extracts | Mycelia growth | | | Plant Extract concentration mean |
|---|---|---|---|---|---|
| | | *M. phaseolina* | *A. flavus* | *F. verticollioides* | |
| ***Erythrophleum suaveolens*** | 10 | 5.87(17.79R) | 4.68(14.91R) | 6.23(20.84R) | 5.59(18.27R) |
| | 20 | 5.33(25.35R) | 3.17(42.36R) | 3.36(57.31R) | 3.95(42.25R) |
| | 30 | 4.51(36.83R) | 1.91(65.27R) | 6.48(17.66R) | 4.30(37.13R) |
| ***Pterocarpus erinaceous*** | 10 | 7.87(9.28S) | 4.08(25.82R) | 7.20(8.51R) | 6.38(6.73R) |
| | 20 | 8.17(12.61S) | 7.12(22.75S) | 7.24(8.01R) | 7.51(8.92S) |
| | 30 | 7.48(4.55S) | 6.07(9.39S) | 6.77(13.98R) | 6.77(1.02R) |
| | Control | 7.14 | 5.50 | 7.87 | 6.84 |

*FLSD (0.05) Plant extract = 0.82; Mycelia growth= 0.55; Plant extract x mycelia growth = 1.42*
*Values in parenthesis represent percentage (%) Reduction (R) or Stimulation (S) of mycelia growth.*

## DISCUSSION

The study showed the effectiveness of the aqueous stem bark extract of *Erythrophleum suaveolens* and *Pterocarpus erinaceous* in the management of fungi associated with sweet potatoleaf spot disease. The stem bark extract of *E. suaveolens*at 10%, 20% and 30% w/v effectively inhibited the mycelia growth of *F. verticollioides* by greater than 50% while it was moderately effective (> 20-50 % inhibition) against the mycelia growth of *Aspergillus flavus* and *M. phaseolina* at 3DAI. At 7DAI, *E. suaveolens* became slightly effective against the test fungi while *P. erinaceous* was not effective against *Aspergillus flavus* and *M. phaseolina*. The fungitoxicity of *E. suaveolens*in this study is similar to that reported by Thippeswamy *et al*. (2013) in which aqueous extracts of *Albizia amara* and *A. saman* at 2mg/ml exhibited strong antifungal activity of greater than 70% against *F. verticoillioides* isolated from maize.

The reduction in the inhibition percentage from 3 DAI to 7DAI may be due to decrease in the fungitoxicity of the aqueous plant extracts with the aging and multiplication of the test fungi. This result is similar to the report of Obani and Ikotun (2021) which noted a gradual decline in growth reduction of *Rhizopus* spp. isolated from melon by *Piper guineense*over time.

In the report of Ekhuemelo *et al*. (2019b), differences in the antifungal activities of the solvent extracts of *Erythrophleum suaveolens* and *Pterocarpus erinaceous* was attributed to differences in the availability of phytochemicals in their stem bark extract. Ekhuemelo *et al* (2019c) identified and characterized Triterpenes such as 21-acetoxylupenone and Betulin ($C_{30}H_{50}O_2$) as the compound responsible for antibacterial activity of *E. suaveolens* stembark against *Ralstonia solanacearum* and *Pseudomonas syringae* causal agent of bacterial wilt and soft rot diseases respectively.

The failure of *Pterocarpus erinaceous* to inhibit the growth of *A. flavus*at 7DAI in this study is in contrast with the report of Obani and Ikotun (2021) which reported 50% reduction of the growth of *A. flavus* by different concentrations of *Xylopia aethiopica*. Nduagu *et al*. (2008) attributed variations in the action of plant extracts to qualitative and quantitative differences in antifungal principles. Ekhuemelo *et al*. (2019d) also reported non activity of *E. suaveolens* compounds against*Serpula lacrymans* and *Sclerotium rolfsii*. Obani and Ikotun (2021) observed that different concentrations of *Xylopia aethiopica* only had effect on *Rhizopus* spp. growth for 2 weeks out of 14 weeks of treatment with botanicals.

Nduagu *et al*. (2008) observed that plant extracts contain phytochemicals that affect microorganisms variably. Ekhuemelo *et al*.(2019a) reported antifungal activity of crude extracts of the stem bark of *E. suaveolens* against *Sclerotium rolfsii*, Aspergillus *fumigatus* and *Rhizopus* spp. and attributed the antifungal activity tothe presence of tannins, moderate

presence of flavonoids, glycosides and saponins. Ekhuemelo *et al*. (2019b) reported high presence of tannins and steroids in the stem bark extract of *Pterocarpus erinaceous* which was able to control *Fomitopsis pinicoca*and *Aspergillus fumigatus* causal agent of stem decay and brown rot decay of wood.This present study revealed theability of the aqueous stem bark extract of *E. suaveolens* and *P. erinaceous* to inhibit the *in vitro* growth of leaf spot pathogens of sweet potato prevalent in the study area.

## CONCLUSION

This study has shown the fungitoxicity of 10%, 20 % and 30 % w/v concentration of the aqueous crude extracts of *E. suaveolens* in the inhibition of the mycelia growth of *M. phaseolina, A. flavus* and *F. verticollioides* associated with the leaf spot disease of sweet potato. The use of *E. suaveolens* stem bark aqueous extract is recommended for the management of the mycelia growth of these fungi *in-vitro*.

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