Assessment of phytochemical constituents of *Erythrophleum suaveolens* (Brenan) stem bark extracts and its biological control of *Aspergillus flavus* isolated from *Zea mays*

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ABSTRACT

This study set out to evaluate the phytochemical composition of *Erythrophleum suaveolens* stem bark extract and its fungicidal attributes towards *Aspergillus flavus* isolated from *Zea mays*. The stem bark extract was obtained using soxhlet extraction method with methanol, chloroform, and water as solvents. Qualitative and quantitative phytochemical analysis was then carried out to determine the concentration of phytochemicals present in the stem bark extracts, this were the tannin, flavonoid, saponin and phenol with a concentration of 2.47, 1.328, 2.72 and 0.838 mg/g respectively. For the antifungal effect, different crude concentrations of the stem bark extracts were then added to agar wells within *Aspergillus flavus* swabbed in a potato dextrose agar plate for 2hrs at room temperature and allowed for diffusion to take place. After 48hrs, the cultured samples were examined for zones of inhibition. The water and chloroform extracts had an inhibitory diameter zone of 10 and 2 mm respectively. The utilization of plant extract of *Erythrophleum suaveolens* could form an alternative to synthetic fungicides/pesticides used in the management of pests and fungi diseases. Significant implication from the result was that, water extract of the plant had higher antifungal potency towards *A. flavus* isolated from *Zea mays*.

Keywords: Antimicrobial, *Aspergillus flavus*, *Erythrophleum suaveolens*, Extracts, Phytochemical
INTRODUCTION

Thousands of secondary metabolites are known to be produced by plants (Quint et al., 2016; Verma et al., 2016; Zhu, 2016). Phytochemicals have been reported to occur in plants’ leaves, stems, roots and seeds, serve as elicitors of defense mechanism in the plants (Wadood et al., 2013). Chlorophyll, proteins and common sugars are examples of primary constituents, while terpenoid, alkaloids, tannin, flavonoid and phenolic compounds are secondary constituents also called bioactive compound (Krishnaiah et al., 2007). The phenolic compounds are a group of plant bioactive components that are omnipresent in nature (Singh et al., 2007). While the biological properties comprise of anti-aging, anti-atherosclerosis, anti-apoptosis, anti-carcinogenic, cardiovascular protection, anti-inflammation with an improvement in endothelial function, as well as an inhibition of angiogenesis with cell proliferation activities (Han et al., 2007). The flavonoids produced by plants are hydroxylated phenol substances, has and have been reported to have antimicrobial effect against some microorganisms and this ability could be attributed to complexity with extracellular and soluble proteins (Marjorie, 1996). On inflammation, saponins had been reported to have an inhibitory effect (Just et al., 1994). Alkaloids are also reported to have cytotoxicity biological properties (Nobori et al., 1994). Terpenoids have been reported to show some vital pharmacological activities as an anti-malarial, anti-inflammatory, antiviral, anticancer, antibacterial activities and inhibition in cholesterol synthesis (Mahato and Sen, 1997). Terpenoids had been reported to attract useful mites and consume insect that are herbivorous in nature (Kappers et al., 2005).

Erythrophleum (Fabaceae- Caesalpinioideae) is a pantropical woody genus and it has been found to be around 15 species found in Africa, Australia and North-East Asia (Duminil et al., 2013). Erythrophleum suaveolens is one of the species of Erythrophleum occurring in Central, West Africa and it survives under a wide range of climatic conditions. These species are common in dry areas, savannahs, semi-evergreen forests and gallery forest from Senegal to Sudan, Kenya, and Zimbabwe (Duminil et al., 2013). The E. suaveolens from Central and West Africa genetic population research conducted by (Liu et al., 2019) reported that there are three subdivided genetic group that exists in allopatry or parapaty. The Erythrophyleum genus phytochemistry had been studied (Hassan et al., 2007). E. suaveolens extract anaesthetic effect on catfish and on gastrointestinal muscle of rabbit jejunum (oryctolague) had been documented (Ogundeko et al., 2014). According to Dongmo et al., (2001) the stem bark extracts of E. suaveolens had an anti-inflammatory and analgesic properties; and possessed an in-vitro antibacterial activity (Aiyegoro et al., 2007).

Toxigenic fungi contamination of agricultural products and food remains a phenomenon that continued to enjoy less attention (Bhat et al., 2010). The Agricultural products with fungal contamination take place at various stages. This can occur during cultivation, harvesting, processing, handling and storage (Devegowda, 2001). The Aspergillus spp are filamentous in nature and they can be found in plant debris, soil and indoor airy environments (Myatt et al., 2008). They can also be isolated from agricultural commodities placed under poor storage conditions (Parra and Magan, 2004). Livestock feed formulated from cereals do serve as source of aflatoxin produced by Aspergillus spp (Pitt and Hocking, 2009). Aspergillus flavus is widely distributed and this result from airborne conidia produced, which can easily be spread by air or insects in a conventional movement (Hedayati et al., 2007). A. flavus grows better in water as a medium between 0.86 and 0.96 (Vujanovic et al., 2001). It can grow in an optimum temperature of 37°C and this contributes to its pathogenicity effect in humans (Hedayati et al., 2007). Maize seeds can be infected by A. flavus in the field prior to their harvest as well as in storage known as storage mold (Klueken et al., 2009).

The study aimed to evaluate the phytochemical constituents of Erythrophleum suaveolens Brenan stem bark extracts (methanol, chloroform and water) and...
assessed its antimicrobial effect on *Aspergillus flavus* isolated from maize seeds.

**MATERIALS AND METHODS**

*Plant materials collection*

The stem bark from a matured tree of *E. suaveolens* was obtained from Adadu village along Ijah Road in Kwali Area Council FCT, Abuja as shown in Fig 1 below and it was identified in National Institute of Pharmaceutical Research and Development (NIPRD), Abuja. The stem bark was then shade dried in crop science lab, Faculty of Agriculture, University of Abuja and after fully air dried, it was crushed to make the fine paste of the dried powdered. Extraction with different solvents like methanol, chloroform and distilled water, were done using soxhlet apparatus. Briefly, for every 200 mL of each solvent, 25 g of the crushed stem bark powder was used for soxhlet extraction. After extraction for 3 consecutive days, the crude liquids were placed in water bath at 55°C for excess solvent evaporation.

![Fig. 1: *E. suaveolens* stem bark](image)

**The *E. suaveolens* stem bark extracts preparation**

**Materials for extraction:** Methanol and Chloroform were procured from Zay-o-Sigma, Soxhlet Extraction Apparatus Heating Unit with Glass Parts JA21830 manufacture by Japson, Whatman® qualitative filter paper, circles, diameter- 90 mm.

**1. Extraction using water**

Twenty-five grams of air-dried finely powdered of *E. suaveolens* stem bark was taken into a conical flask with 200ml distilled water and the mixture was heated on a hot plate set at 30º-40ºC for 20min with continuous stirring. The filtrate from the water extract was then obtained by filtration through a filter paper (11 μm (Particle retention) and preserved in refrigerator prior to its use.

**2. Solvent Extraction**

*E. suaveolens* crude stem bark extract was prepared by using soxhlet extraction principle. Twenty-five grams of an air-dried stem bark powdered of *E. suaveolens* in a thimble with 250ml of solvents such as methanol and chloroform separately for extraction. The process was set for 24 hours till the solvent in siphon tube turns colorless and this was heated at 30-40ºC to evaporate the solvent.
and was stored in refrigerator at 4ºC for further phytochemical analysis.

**Phytochemicals qualitative analysis of E. suaveolens stems barks**
The method used by Sofowora, (1993), was used in testing for the presence of bioactive compounds in the stem bark extracts as follows:

**Carbohydrates testing**
An equal volume of Fehling A and Fehling B reagents of 2ml added to the crude stem bark extract, mixed and put up to heat to a boiling point. A red brick precipitate formed which indicate the presence of reducing sugars.

**Saponins testing**
The crude extract of stem bark and 5ml of distilled water mixed thoroughly and stable foam was seen as an indication for the presence of saponins.

**Phenols and tannins test**
A 2% solution of FeCl₃ was mixed with the crude stem bark extract and a blue-green colour appeared indicating the presence of phenols and tannins.

**Flavanoid test**
The crude stem bark extract was mixed in a 2% solution of NaOH which produced an intense yellow colour and turned colourless on addition of few drops of diluted acid indicated the presence of flavonoids

**Steroid test**
Approximately, 2ml of chloroform mixed with crude stem bark extract with addition of 2ml concentrated H₂SO₄ in the lower chloroform layer shows a red colour indicating the presence of steroids.

**Terpenoids test**
The crude stem bark extract was dissolved in 2ml of chloroform and evaporated to dryness and 2ml conc. H₂SO₄ added and heated for about 2 minutes to give a grayish colour indicating the presence of terpenoids.

**Alkaloid test**
Crude stem bark extract mixed in 1% HCl and heated with Mayer’s and Wagner’s reagents and a turbidity precipitate indicating the presence of alkaloids.

**Volatile oil test**
Hydro- distillation of the crude stem bark extract and the distillate collected in a well assembly graduate tube shows an aqueous portion and was automatically separated from the volatile oil.

**Cardenolides test**
Approximately, 2ml of benzene and 1ml of the crude stem bark extract mixed and a turbid brown colour formed indicating the presence of cardenolides.

**Cardiac Glycosides test**
Approximately 0.5g of the crude stem bark extract was dissolved in 2ml glacial acetic acid with a drop of ferric chloride solution to downplay with 2ml of concentrated sulphuric acid which formed a brown ring indicating the presence of deoxy sugar characteristic of cardiac glycosides.

**Phlobatannins testing**
A few drops of 1% HCl to 1ml of crude stem bark extract and was brought to a boiling point. A reddish precipitation formed indicating the presence of phlobatannins.

**Antifungal activity Determination**
Swabbing using sterile cotton swabs in a Potato Dextrose Agar (PDA) plate with 8hrs old broth culture of the fungus Aspergillus flavus and a sterile cork borer used in making wells at the center of the culture plate. Then, 0.6g of dried extracts was dissolved in 2ml of Dimethyl Sulfoxide (DMSO) each and used as samples. In this experiment the Dimethyl Sulfoxide (inert organic solvent) and Fluconazole (10µg, antifungal drug) was used as negative control and positive control, respectively. The Plant extracts (water, chloroform and methanol) samples were used at crude concentration without serial dilution. The plant extract was added into each well using a sterilized micropipette and allowed the extracts for diffusion for 2hrs at room temperature and then incubated at 28ºC for 48hrs.
RESULTS AND DISCUSSION
In this study from Table 1, the phytochemical qualitative analysis shows that the *E. suaveolens* stem bark extracts contains tannins, steroids, phenols, terpenoids, saponin, flavonoid, phlobatannins and cardiac glycosides. The phytochemical quantitative analysis from Table 2 shows the presence of tannins at a concentration of 2.47 mg/g, flavonoids at 1.328 mg/g, saponin at 2.72 mg/g, phenols at 0.838 mg/g. These phytochemicals have been linked to antimicrobial activities of some plants (Njoki et al., 2017; Murugan et al., 2013).

### Table 1: Qualitative analysis of *Erythrophleum suaveolens*

<table>
<thead>
<tr>
<th>Parameter</th>
<th><em>Erythrophleum suaveolens</em> stem bark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>-</td>
</tr>
<tr>
<td>Phenols</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
</tr>
<tr>
<td>Cardenolids</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>+</td>
</tr>
<tr>
<td>Saponin</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>+</td>
</tr>
<tr>
<td>Balsams</td>
<td>-</td>
</tr>
<tr>
<td>Volatile oils</td>
<td>-</td>
</tr>
<tr>
<td>Phlobatannins</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Cardenolides</td>
<td>-</td>
</tr>
</tbody>
</table>

In most African countries, the ethno-pharmacological use of plants is prevalent in combating infectious diseases (Tagoe et al., 2009). Chemical compounds are naturally a defensive mechanism present in several plants and microbial tissues which comprises of antibiotics, phenolic compounds, alkaloids, terpenes and proteins (Ahmadi et al., 2010; Souza et al., 2010).

### Table 2: Quantitative analysis of *Erythrophleum suaveolens*

<table>
<thead>
<tr>
<th>Parameter</th>
<th><em>Erythrophleum suaveolens</em> stem bark (mg/g)</th>
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<tbody>
<tr>
<td>Tannins</td>
<td>2.47</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>1.328</td>
</tr>
<tr>
<td>Saponin</td>
<td>2.72</td>
</tr>
<tr>
<td>Phenols</td>
<td>0.838</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Nil</td>
</tr>
</tbody>
</table>
Aspergillus flavus are ubiquitous, saprophytic, and regarded as a weak parasite (Hua, 2013) and also considered as a toxigenic fungal colonizer of fruits and cereals produces amycotoxins as seen in aflatoxin from a food safety perspective (Tejero et al., 2021). For this study, the biological control of A. flavus using different stem bark extract of E. suaveolens was investigated and as shown in Fig. 1, the growth of A. flavus was inhibited by water extract of E. suaveolens and also by the chloroform extract. The methanol extract did not inhibit the A. flavus growth. The bioactive compounds determined in this study have been shown to agree with the cause of antimicrobial activities reported in other studies through various mechanisms (Miron et al., 2014; Handali et al., 2013; Shobowale et al., 2013).

In some other plant extracts studies, E. suaveolens has appreciable high quantity of bioactive secondary metabolites that are significant excellent in wood protection properties against fungi. Tagoe et al., (2009) in his studies investigated the antimicrobial properties of garlic, ginger and lime against A. flavus using ethanol and water for the extracts and found out that water base extraction technique was the most effective in inducing antifungal properties of lime while ethanol base extract was the best for ginger. Garlic on the other hand was said to have exhibited a good antifungal property in both ethanol and water extraction media. Souza et al., (2011) reported that stem bark extract from Spirulina platensis showed antifungal activity against Aspergillus flavus. Furthermore, in this study from Table 3, the water extract of E. suaveolens inhibit the growth of A. flavus with an inhibition zone diameter of 12mm, for the organic extract of chloroform and methanol, the chloroform extract inhibited the growth of A. flavus with an inhibition zone

### Table 3: Erythrophleum suaveolens extracts antifungal effect on Aspergillus flavus in terms of diameter of clearance (millimeter mm) with agar diffusion method after 48hrs incubation at 28°C

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Inhibition zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>12</td>
</tr>
<tr>
<td>Chloroform</td>
<td>2</td>
</tr>
<tr>
<td>Methanol</td>
<td>0</td>
</tr>
<tr>
<td>Negative Control (DMSO)</td>
<td>0</td>
</tr>
<tr>
<td>Positive control (fluconazole)</td>
<td>19</td>
</tr>
</tbody>
</table>
diameter of 2mm, the methanol extract did not inhibit the growth of *A. flavus*. In this study, with the fluconazole as positive control and inhibitory zone diameter of 19mm and Dimethyl Sulfoxide (DMSO) the negative control did not show any inhibitory effects to the growth of *A. flavus*.

**CONCLUSION**
The study achieved the use of the best extraction solvent for the extraction of phytochemicals from *Erythrophleum suaveolens* stem bark and its fungicidal attributes towards *Aspergillus flavus* isolated from *Zea mays*. The results obtained shows that using methanol as extraction solvent results in the maximum extraction of tannin, flavonoids and saponin. Furthermore, the concentration of flavonoids, saponin and tannins obtained correlates very well with the anti-microbial activity as reported in the previous works by (Miron et al., 2014; Handali et al., 2013; Shobowale et al., 2013). Chloroform results in the least extraction of various phytochemicals, may be due to poor solubility of these phytochemicals in chloroform. Meanwhile the water extracts of *E. suaveolens* stem bark showed higher biological control to the growth of *Aspergillus flavus* among the three extracts investigated in this study.

**Author’s Contribution**
YUSUF, Hajara O.: Carried out the experimental work, wrote and edited the submitted manuscript
OLU, Joshua: Edited the first draft of the manuscript and the data analysis
Charity Irekpita AKHIGBE: Air dried the *E. suaveolens* stem bark and prepared it into powdered for extraction and monitored the extraction processes in the laboratory.

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Our Appreciation to Mrs. Joy Adaeze Alu for her great assistance while carrying out the experimental work in crop science laboratory, University of Abuja and Mr. Musa Liman Zubairu who helped in getting the stem bark from Adadu village in Kwali area council, Abuja.

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