Inhibitory Activity of Plant Extracts on Aflatoxin B₁ Biosynthesis by Aspergillus flavus

S. Thippeswamy¹, D. C. Mohana¹*, R. U. Abhishek¹, and K. Manjunath¹

ABSTRACT

The inhibitory activities of aqueous and solvent extracts of twelve selected medicinal plants were evaluated against biosynthesis of aflatoxin B₁ (AFB₁) by Aspergillus flavus. The A. flavus was isolated from maize, and aflatoxin B₁ biosynthesis was confirmed by comparison with standard AFB₁ using TLC method. In vivo antiaflatoxigenic efficacies of activity guided solvent extracts were determined in maize model system. All the extracts showed varying degree of antifungal and AFB₁ inhibitory activities, but chloroformic extract of Albizia amara, Cassia spectabilis and Solanum indicum, and methanolic extract of Acacia catechu, Albizia saman and Anogeissus latifolia showed the highest activity. Further investigations on identification of active principles from these plants are needed to develop plant based formulations for management of A. flavus growth and AFB₁ contamination in food grains.

Keywords: Antiaflatoxigenic, Maize, Plant extracts.

INTRODUCTION

Fungal deteriorations and mycotoxin contamination of various food and feedstuffs are a major problem in the tropics and subtropics, where climatic conditions and storage practices are favourable to fungal growth (Quiroga et al., 2009; Shukla et al., 2009; Salari et al., 2012). The risk of mycotoxins, particularly aflatoxins contamination is an important food safety concern for grains and other field crops worldwide (Kumar et al., 2007; Reddy et al., 2009). The Food and Agriculture Organization (FAO) estimated that around 25% of the world’s cereals are contaminated by mycotoxins, including aflatoxins (Dowling, 1997). Aflatoxin B₁ is one of the most common and dangerous mycotoxin produced by A. flavus (Manafi and Khosravinia, 2013). Aflatoxins are found in a variety of food commodities such as maize, ground nut, cotton seeds, and other cereals worldwide, and it is reported that about 4.5 billion people in developing countries are systematically exposed to uncontrolled amounts of aflatoxins (Shukla et al., 2008).

The physical (aeration, cold storage, rapid drying, and radiation) and chemical (food preservatives and pesticides) treatments are commonly used to control the deterioration and aflatoxins contamination of food grains by A. flavus (Passone et al., 2008). Most of these control strategies are costly, health hazardous, and not affordable to rural subsistence farmers (Shukla et al., 2009). Further, residues of these synthetic chemicals in agricultural produce, products, and their by-products cause damage to the health of animals and humans (Deng et al., 2011). Due to these, the use of natural products to control the mould and mycotoxins contamination in cereal grains, have attracted the attention of the scientists to search some newer agents from plants that inhibit aflatoxins biosynthesis. Such products of higher plants would be

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biodegradable, renewable in nature, and safe to human health (Verma and Dubey, 1999). Different crude extracts of plant materials rich in polyphenolics and alkaloids are becoming important in food industries because of their antifungal and antiaflatoxigenic activities. Hence, such plants extracts could potentially be used to control mycotoxicogenic fungi in foods and feeds, and for avoiding the use of synthetic chemicals. Considering these, we have screened 48 plants preliminarily for their inhibitory activity against A. flavus, among which 12 plants showed significant activity. Hence, these plants were selected for further investigations on inhibition of AFB$_1$ biosynthesis, and the obtained results are presented in this paper.

**MATERIALS AND METHODS**

**Chemicals and Culture Media**

The Sabouraud Dextrose Agar/Broth (SDA/SDB) and Dimethyl sulfoxide (DMSO) were purchased from Hi-Media, Mumbai (India). Mancozeb 75% WP (dithane M-45) was obtained from Indofil chemicals, Mumbai (India). All solvents, reagents and iodo-nitro-tetrazolium (INT) were procured from Sisco Research Laboratory, Mumbai (India). Microtiter plates (96 wells) and serological pipettes were purchased from Axiva, New Delhi (India). The standard aflatoxin B$_1$ (AFB$_1$) was obtained from Sigma, Germany and Silica gel 60 F$_{254}$ coated preparative aluminium Thin Layer Chromatography (TLC) plates (20×20 cm) from Merck, Darmstadt (Germany).

**Plant Materials**

Fresh disease free leaves of 12 different medicinal plant species were collected from southern part of Karnataka, India. The plant samples were authenticated by Dr. Seetharam, Professor, Department of Biological Sciences, Bangalore University and the authenticated voucher specimens have been deposited at the Herbarium centre, Department of Microbiology and Biotechnology, Bangalore University, Bangalore (Table 1).

**Table 1. Antifungal activity of aqueous extract of selected medicinal plants against aflatoxigenic A. flavus at 10% concentration.**

<table>
<thead>
<tr>
<th>Plants</th>
<th>Voucher number</th>
<th>Activity (% mycelial inhibition)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acacia catechu</em> (L.f.) Willd.</td>
<td>BUB-MB and BT-DCM-JU10-25</td>
<td>18.3±0.91$^a$</td>
</tr>
<tr>
<td><em>Acacia ferruginea</em> DC.</td>
<td>15</td>
<td>12.8±0.72</td>
</tr>
<tr>
<td><em>Adenanthera pavonina</em> L.</td>
<td>61</td>
<td>12.6±0.68</td>
</tr>
<tr>
<td><em>Albizia amara</em> (Roxb.) B.Boivin</td>
<td>23</td>
<td>30.8±1.42</td>
</tr>
<tr>
<td><em>Albizia odoratissima</em> (L.f.) Benth.</td>
<td>55</td>
<td>14.5±0.85</td>
</tr>
<tr>
<td><em>Albizia saman</em> (Jacq.) Merr.</td>
<td>33</td>
<td>29.3±1.36</td>
</tr>
<tr>
<td><em>Anogeissus latifolia</em> (Roxb. ex DC.) Wall.</td>
<td>24</td>
<td>22.3±1.12</td>
</tr>
<tr>
<td><em>Caesalpinia coriaria</em> (Jacq.) Willd.</td>
<td>44</td>
<td>11.7±0.66</td>
</tr>
<tr>
<td><em>Cassia spectabilis</em> DC.</td>
<td>38</td>
<td>28.6±1.06</td>
</tr>
<tr>
<td><em>Dodonaea viscosa</em> Jacq.</td>
<td>11</td>
<td>11.5±0.72</td>
</tr>
<tr>
<td><em>Prosopis juliflora</em> (Sw.) DC.</td>
<td>12</td>
<td>15.6±0.87</td>
</tr>
<tr>
<td><em>Solanum indicum</em> L.</td>
<td>16</td>
<td>42.4±1.45</td>
</tr>
</tbody>
</table>

$^a$ Data given are mean of four replicates; media impregnated with the same amount of water served as control.
Preparation of Aqueous Extracts

The aqueous extracts of 12 plant species were prepared following the procedure of Mohana et al. (2007). Briefly, 50 g of thoroughly washed and blot dried plant material was macerated separately with 100 mL sterile distilled water in a warrior blender for 10 minutes. The macerate was filtered through double-layered muslin cloth, centrifuged at 4,000×g for 30 minutes and again filtered the supernatant through Whatman No. 1 filter paper, and sterilized at 121°C for 20 minutes. The obtained extracts were considered as 100% and 10% of each extract impregnated SDA was used for antifungal activity assay.

Preparation of Solvent Extracts

The successive solvents extracts of 12 plant species were prepared following the procedure of Thippeswamy et al. (2011). Briefly, 50 g powder of each shade dried plants were filled in the thimble separately and extracted successively with 200 mL of petroleum ether, toluene, chloroform, methanol and ethanol using a soxhlet extractor. The residual solvents in the extracts were removed using rotary flash evaporator. The dried plant extracts were re-suspended in DMSO and subjected to antifungal and aflatoxicogenic activities at different desired concentrations.

Antifungal Activity Assay

Isolation of AFB₁ Producing A. flavus from Maize

A total of 45 strains of A. flavus were isolated from 25 maize varieties, and AFB₁ producing A. flavus strains were detected by methyl-β-cyclodextrin enriched culture media (Rahimi et al., 2008). The AFB₁ content was qualitatively analysed by TLC method and quantitatively by spectrophotometric methods (Shukla et al., 2008). The A. flavus MY5 strain was able to produce the highest concentration of AFB₁ and was selected as a test organism for determining the antifungal and antiaflatoxicogenic efficacies.

Poisoned Food Technique

Aqueous and successive solvent extracts of all 12 plants were subjected to antifungal activity assay by poisoned food technique following the procedure of Mohana et al. (2010). Briefly, requisite concentrations of all the test samples were incorporated separately into SDA medium (10% in case of aqueous extracts and 0.031 to 4 mg mL⁻¹ in case of solvent extracts), autoclaved, poured into Petri dishes (20 mL plate⁻¹) and allowed to cool. Five millimetre disc of 7-day-old culture of A. flavus was placed at the centre of the Petri dishes. The plates were incubated at 28±1°C for 7 days. The media containing DMSO served as a negative control for solvent extracts and dithane M-45 served as a positive control. Four replicates were maintained for each concentration. The fungi-toxicity of the extract in terms of percentage inhibition (I%) of mycelial growth was calculated using the following formula:

\[ I\% = \frac{(dc-dt) \times 100}{dc} \]

Where, \( dc \) = Average diameter of mycelial growth in the control, \( dt \) = Average diameter of mycelial growth in the treatment.

Determination of MIC by Broth Microdilution Method

The broth microdilution method was used to determine the minimum inhibitory concentrations (MIC) of activity guided solvent extracts following the procedure of Hajji et al. (2010). Briefly, 200 µL of two-fold serially diluted of each extract (0.031 to 4 mg mL⁻¹) in SDB were added separately to the 96-well microtiter plate and inoculated with 15µL of A. flavus spore suspension containing \( 10^{4} \) spores mL⁻¹ and incubated at 30°C for 72 hours. DMSO
served as a negative control and dithane M-45 was used as a positive control. After incubation, the MIC values of the extracts were detected by the addition of 50 μL of INT (2 mg mL\(^{-1}\) in water). The colourless tetrazolium salt acts as an electron acceptor and is reduced to a red-coloured formazan product by biologically active organisms. Where fungal growth was inhibited, the solution in the well remained clear after incubation with INT. The color intensity was measured using microtiter plate reader (ELx800, Bio-Tek Instruments, US). MIC was defined as the lowest concentration at which no visible fungal growth was observed.

**In vitro and In vivo Efficacies of Activity Guided Solvent Extracts on AFB\(_1\)**

**Biosynthesis by A. flavus**

**In vitro Assay**

The in vitro efficacies of activity guided solvent extracts on AFB\(_1\) production in maize seeds were determined following the procedures of Shukla et al. (2008). Briefly, 100 μL of a spore suspension (10\(^4\) spores mL\(^{-1}\)) of A. flavus was inoculated into SMKY broth containing the requisite amount of active solvent extracts (0.0312 to 2.0 mg mL\(^{-1}\)) and incubated at 28±2°C for 10 days. The flask containing medium without extract served as a negative control and dithane M-45 was used as a positive control. After incubation, the broth cultures were filtered through Whatman No. 1 filter paper and the filtrate was used for the isolation of AFB\(_1\) by adding an equal volume of CHCl\(_3\). The CHCl\(_3\) layer was separated and passed through anhydrous Na\(_2\)SO\(_4\) and allowed to evaporate in dark condition at 28±2°C. The residue was re-dissolved in 1 mL of CHCl\(_3\) and 10 μL of sample was spotted on the TLC plate adjacent to AFB\(_1\) standard. The plates were developed in CHCl\(_3\)-acetone (96:4) solvent system, air-dried and visualized under ultra-violet (360nm) light (UV-cabinet, Labline, India). Qualitative identification of AFB\(_1\) content was done by visual comparison of intensity of fluorescence of the samples with AFB\(_1\) standard spots. For quantitative estimation, the fluorescent spots were scraped out from the plates, dissolved in 5 mL cold CH\(_3\)OH, and centrifuged at 3,000 rpm for 5 minutes. The absorbance of supernatant was measured at 360 nm using a spectrophotometer (ELICO SL-210, India) and AFB\(_1\) content was calculated using the following formula:

\[
\text{AFB}_1 \text{ content (μg L}^{-1}) = \text{(DXM/EXL)} \times 1000
\]

Where, \(D\) = Absorbance; \(M\) = Molecular weight of AFB\(_1\) (312); \(E\) = Molar extinction coefficient of AFB\(_1\) (21,800) and, \(L\) = Path length (1 cm cell)

**In vivo Efficacy**

The in vivo efficacies of active solvent extracts on AFB\(_1\) production in maize seeds were determined following the procedures of Garcia et al. (2012) with some modifications. Briefly, freshly harvested maize samples were collected, surface sterilized under UV, and the water activity (a\(_w\)) was adjusted to 0.95 by adding sterile distilled water. The maize samples were treated with requisite concentrations (0.0312 to 2.0 mg mL\(^{-1}\)) of activity guided solvent extracts separately and inoculated with 100 μL of a spore suspension (10\(^4\) spores mL\(^{-1}\)) of A. flavus. All treatments were separately stored in plastic containers (200 g pack\(^{-1}\)) and incubated at 25±2°C for up to 15 days. After incubation, the milled maize seeds were subjected to AFB\(_1\) extraction and quantification (Singh et al., 1991; Shukla et al., 2008).

The percent incidence of A. flavus in the treated and untreated samples was determined by standard blotter method (ISTA, 1996) and seedling vigour index (SVI) was analysed using the following formula (Sparg et al., 2005):

\[
\text{SVI}= (\text{Mean of root length} + \text{Mean of shoot length}) \times \text{Percentage of seed germination}
\]
**Statistical Analysis**

All experiments were performed in four replicates and values were expressed as means ± standard error. Analysis of variance was conducted, and the differences between values were tested for significance by ANOVA with the SPSS 20 (IBM, USA) programme. Differences at \( P \leq 0.05 \) were considered statistically significant.

**RESULTS AND DISCUSSION**

The recent intensive works have revealed that the plants are important source for the development of potentially useful eco-friendly fungicides. *In vitro* evaluations are the first step towards this goal. In this study, we have screened the aqueous extracts of 12 plants viz., *Acacia catechu*, *A. ferruginea*, *Adenanthera pavonina*, *Albizia amara*, *A. odoratissima*, *A. saman*, *Anogeissus latifolia*, *Caesalpinia coriaria*, *Cassia spectabilis*, *Dodonaea viscosa*, *Prosopis juliflora* and *Solanum indicum* belonging to seven families for their antifungal efficacy in terms of percent mycelial inhibition against aflatoxigenic *A. flavus* at 10% concentration by poisoned food technique. All the plants showed varying degree of inhibitory activities with the percent mycelial inhibition ranging from 11.5 to 42.4% (Table 1). The highest percent mycelial inhibition was observed in *S. indicum*, whereas the least inhibition was observed in *D. viscosa*.

The antifungal activity of the desired different concentrations of five successive solvent extracts of each plant was determined against *A. flavus* by poison food technique for determination of percent mycelial inhibition and broth microdilution method for determination of MIC. The obtained results are presented in Table 2. The highest mycelial inhibition of *A. flavus* was observed in chloroformic extract (CE) of *A. pavonina*, *A. amara*, *C. spectabilis* and

**Table 2.** Inhibitory activities of activity guided solvent extracts of selected medicinal plants on AFB\(_1\) biosynthesis and *A. flavus* growth.

<table>
<thead>
<tr>
<th>Plant names</th>
<th>Extracts(^a)</th>
<th>% mycelial inhibition (2 mg mL(^{-1}))</th>
<th>MIC (mg mL(^{-1}))</th>
<th>AFB(_1) content(^b)</th>
<th>In vitro (µg L(^{-1}))</th>
<th>In vivo (µg kg(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. catechu</em></td>
<td>M</td>
<td>26.2±0.43(^c)</td>
<td>1.0</td>
<td>300±11</td>
<td>850±18</td>
<td></td>
</tr>
<tr>
<td><em>A. ferruginea</em></td>
<td>M</td>
<td>23.8±0.16</td>
<td>1.0</td>
<td>380±12</td>
<td>925±22</td>
<td></td>
</tr>
<tr>
<td><em>A. pavonina</em></td>
<td>C</td>
<td>19.6±0.56</td>
<td>1.5</td>
<td>575±16</td>
<td>1325±27</td>
<td></td>
</tr>
<tr>
<td><em>A. amara</em></td>
<td>C</td>
<td>59.0±0.47</td>
<td>0.5</td>
<td>0</td>
<td>250±12</td>
<td></td>
</tr>
<tr>
<td><em>A. odoratissima</em></td>
<td>M</td>
<td>22.6±0.24</td>
<td>1.0</td>
<td>450±14</td>
<td>975±24</td>
<td></td>
</tr>
<tr>
<td><em>A. saman</em></td>
<td>M</td>
<td>57.8±0.72</td>
<td>0.5</td>
<td>0</td>
<td>250±14</td>
<td></td>
</tr>
<tr>
<td><em>A. latifolia</em></td>
<td>M</td>
<td>27.8±0.25</td>
<td>0.5</td>
<td>250±8</td>
<td>650±16</td>
<td></td>
</tr>
<tr>
<td><em>C. coriaria</em></td>
<td>M</td>
<td>18.6±0.52</td>
<td>1.0</td>
<td>450±12</td>
<td>925±23</td>
<td></td>
</tr>
<tr>
<td><em>C. spectabilis</em></td>
<td>C</td>
<td>42.4±0.37</td>
<td>0.5</td>
<td>100±6</td>
<td>425±15</td>
<td></td>
</tr>
<tr>
<td><em>D. viscosa</em></td>
<td>M</td>
<td>14.3±0.42</td>
<td>2.0</td>
<td>510±15</td>
<td>1425±28</td>
<td></td>
</tr>
<tr>
<td><em>P. juliflora</em></td>
<td>M</td>
<td>22.2±0.36</td>
<td>1.5</td>
<td>400±12</td>
<td>1010±25</td>
<td></td>
</tr>
<tr>
<td><em>S. indicum</em></td>
<td>C</td>
<td>63.5±0.56</td>
<td>0.25</td>
<td>0</td>
<td>175±12</td>
<td></td>
</tr>
<tr>
<td>Negative control</td>
<td>-</td>
<td>0</td>
<td></td>
<td>1500±20</td>
<td>2000±32</td>
<td></td>
</tr>
<tr>
<td>Dithane M-45</td>
<td>-</td>
<td>54.6±0.32</td>
<td>0.5</td>
<td>50±3</td>
<td>NC(^d)</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) P: Petroleum ether extract; C: Chloroformic extract; M: Methanolic extract, DMSO served as negative control.

\(^b\) 2 mg mL\(^{-1}\) for *in vitro* treatment and 2 g kg\(^{-1}\) for *in vivo* treatment.

\(^c\) Data given are mean of four replicates±standard error.

\(^d\) NC: Not Checked.
S. indicum, and methanolic extract (ME) of A. catechu, A. saman and C. coriaria with the percent mycelial inhibition ranging from 14.3 to 63.5% and MIC ranging from 0.25 to 2.0 mg mL\(^{-1}\), depending on plant species. The S. indicum (CE) showed highest percent mycelial inhibition with the least MIC, whereas D. viscosa showed the least percent of mycelial inhibition with the highest MIC. On comparative evaluation with synthetic fungicide dithane M-45, the activity of A. amara (CE), A. saman (ME), C. spectabilis (CE), and S. indicum (CE) was comparable to the positive control dithane M-45. The present findings confirm that the chloroform and methanol are the best solvents for the isolation of bioactive compounds from the respective plants.

In vitro and in vivo inhibitory activities of active solvent extracts on AFB\(_1\) biosynthesis by A. flavus were determined qualitatively by TLC method and quantitatively by spectrophotometric method. The results were presented in Table 2. In the negative control, AFB\(_1\) production was 1,500 \(\mu\)g L\(^{-1}\) in vitro and 200 \(\mu\)g kg\(^{-1}\) in vivo. The A. amara (CE), A. saman (ME) and S. indicum (CE) were completely inhibited the AFB\(_1\) production in vitro at 2 mg mL\(^{-1}\). Similarly, the AFB\(_1\) biosynthesis was significantly inhibited by all of the plant species at 2 g kg\(^{-1}\) with decreased AFB\(_1\) content ranging from 175 to 1425 \(\mu\)g kg\(^{-1}\), depending on plant species. The percent incidence of A. flavus in maize samples of the control set was 100%, whereas, the percent incidence of A. flavus was greatly decreased in S. indicum (18.9%) followed by A. amara (22.5%) and A. saman (30.7%) treated maize (Figure 1). The present study confirms that the A. amara (CE), A. pavonina (CE), C. spectabilis (CE), S. indicum (CE), A. catechu (ME), A. ferruginea (ME), A. odoratissima (ME), A. saman (ME), A. latifolia (ME), and P. juliflora (ME) are effective extracts for inhibiting AFB\(_1\) biosynthesis.

A survey of the literature reveals that the extracts of A. catechu have significant antibacterial and antifungal activities (Bhardwaj and Laura, 2009; Das \textit{et al.}, 2011; Joshi \textit{et al.}, 2011; Negi and Dave, 2010). Also, the antimicrobial and antioxidant activities of crude extracts of A. amara and A. saman against human and plant pathogenic bacteria and fungi have been reported (Raghavendra \textit{et al.}, 2008; Prasad \textit{et al.}, 2008; Azhar \textit{et al.}, 2009;

![Figure 1](https://example.com/figure1.png)

**Figure 1.** In vivo efficacy of activity guided solvent extracts of some selected plants on percent incidence of A. flavus in maize model system. (Data given are mean of four replicates±standard error; DMSO served as a negative control).
Effect of Plant Extracts on AFB1 Biosynthesis  ___________________________________  
Nnamdi et al., 2010; Arulpriya et al., 2010; Ferdous et al., 2010; Praveen et al., 2011; Thippeswamy et al., 2011; Karmegam et al., 2012; Ajam et al., 2012). Other researchers have reported the anti-inflammatory, cytotoxic, and antibacterial activities of A. pavonina (Ahmed et al., 2012, Ara et al., 2010; Hussain et al., 2011; Mahida et al., 2007), the antimicrobial activity of the extract of A. ferruginea, A. odoratissima, A. latifolia, C. coriaria, C. spectabilis, D. viscosa, S. indicum, (Hishobkar et al., 2010; Sangetha et al., 2008; Ashokkumar et al., 2012; Pirzada et al., 2010; Siva et al., 2011), and the antifungal activity of P. juliflora against some storage moulds (Satish et al., 2007; Ikram and Dawar, 2013). To the best of our knowledge, there are no reports available on the inhibitory activity of these plants on aflatoxin B1 biosynthesis from A. flavus. In the present investigation, the antiaflatoxigenic activity of these plants has been demonstrated for the first time.

The efficacy of the plant extracts over the commonly used synthetic fungicide dithane M-45 at the lowest levels of MIC with no adverse effect of treatments on seed germination with enhanced seedling growth was observed. It confirms that the collective effect of phyto-constituents of extracts may be responsible for the enhanced seedling growth. Based on the antifungal activity, the crude plant extracts could be recommended as plant-based preservatives for prevention of moulds growth and aflatoxin contamination in cereals as well as for protecting crops against fungal pathogens. This is a preliminary investigation; further studies on organoleptic parameters, and toxicological and phytochemical studies are needed before final recommendation.

CONCLUSIONS

The results of these investigations suggest that the extracts of A. amara, A. saman, C. spectabilis and S. indicum are more effective on inhibition of A. flavus growth and aflatoxin B1 biosynthesis Than other plant extracts tested. Hence, these plants could be used for the development of natural fungicides for management of post harvest fungal infestation and mycotoxin contamination in food commodities after toxicological studies.

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**Effect of Plant Extracts on AFB1 Biosynthesis**


فعالیت بازدارنده‌گی عصاره گیاهان روی تولید افلاتوکسین ب1 توسط Aspergillus flavus

س. تیپسواهی، د. س. موهنا، ر. ی. آیهیشک، و ک. مانجونات

چکیده

اثر بازدارنده‌گی عصاره‌های محلول در آب با در حالی از 12 گیاه دارویی منتخب روی تولید افلاتوکسین ب1 (AFB1) توسط (Aspergillus flavus) ارزیابی شد. AFB1 هم با مقایسه با استاندارد و کاربرد روش اصلی AFB1 در محیط زندگی، نتیجه بخش بودن اثر ضد افلاتوکسینی عصاره‌های محلول در حال مناسبی هدایت فعالیت (activity guided) فعالیت را نشان دادند و در پی عصاره کلامپرومیک ب1 را نشان دادند. ویل عصاره کلامپرومیک Albizia amara، Solanum indicum و Cassia spectabilis و عصاره زیستی در مناطقی Acacia catechu و Albizia saman و Anogeissus latifolia و به ترتیب فعالیت را نشان دادند. بررسی های بیشتر روی شناسایی ماده اصلی این گیاهان مورد نیاز است تا پیوند فرمولاسیون‌های گیاه-پایه برای AFB1 و آلودگی‌های اصلی A. Flavus مدیریت رشده دست آورده.