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# Phytochemical Profile and Evaluation of the Allelopathic Effect of the Aqueous Extract of *Fimbristylis miliacea* (L.) Vahl (Cyperaceae)

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**Abstract**

The allelopathic potential of some plant species has been scientifically proven over the years. The use of such natural compounds with phytotoxic activity is an alternative to synthetic herbicides. Thus, this research aims to analyze the phytochemical profile and the allelopathic effect of aqueous extracts of *Fimbristylis miliacea* (L.) Vahl. The allelopathic potential was evaluated by germination bioassays using seeds from two receptor species (*Lactuca sativa* L. and *Emilia fosbergii* Nicolson) and seven concentrations of aqueous extracts of *F. miliacea* aerial and underground parts in triplicate. The variables evaluated were germination (G) and germination speed index (GSI). The data obtained were submitted to F test and the averages to regression analysis. In addition, phytochemical analyses were performed to analyze possible allelochemicals present in aqueous extracts of *F. miliacea* through phytochemical screening and by high performance liquid chromatography (HPLC). The aqueous extracts of aerial and underground parts of *F. miliacea* inhibit the germination of *E. fosbergii* seeds at all concentrations analyzed (0.94, 1.87, 3.75, 7.5, 15 and 30%). However, the same extracts do not show any effects when evaluated in *L. sativa* seeds. The aqueous extract of *F. miliacea* shows important chemical constituents (gallic acid, chlorogenic acid, rutin, luteolin, apigenin, acacetin, and alkaloids) that can be directly related to the allelopathic effects observed in *E. fosbergii* seeds.

**Keywords:** allelochemicals, weed, phytotoxicity

## 1. Introduction

Allelopathy can be understood as a form of plant interference that significantly influences the development dynamics of other susceptible plants. Some factors are included in this process, such as allelochemical biosynthesis, the release of these compounds into nature, environmental conditions, and their interaction (Trezzi et al., 2016). According to Wardle et al. (2011), an allelochemical produced by some species can provide multiple ecological functions considering that such chemical compounds are highly active, and their effects depend on specific environmental conditions.

Allelochemicals isolated from plant species offer a vast, virtually untapped reservoir of chemical compounds with several applications. An example is the use of these substances in agriculture to control pests or weeds with less toxic effects compared to synthetic compounds, which are toxicologically and environmentally undesirable (Li et al., 2010). Many allelochemicals extracted from plants have been gaining importance as pest control agents that spread easily in economically important crops. Such compounds are capable of suppressing the germination and growth of several weeds, some of which are resistant to herbicides obtained from toxic synthetic products (Sangeetha and Baskar, 2015).

Jabran et al. (2015) reinforced that a practical control of weeds can be achieved by using techniques related to allelopathy. It is important to note that this type of control does not harm the environment or increase the costs of pest control on rural properties, and that it can be applied as a unique strategy in certain cultivation systems, such as organic agriculture. In this way, the structure of allelochemicals can be used as a basis for the synthesis of new pesticides. Such biopesticides may be less harmful to the environment compared to synthetic agrochemicals (Amb and Ahluwalia, 2016).

Several plants have allelopathic effects and are accessible to humans in various parts of the world. The Cyperaceae family, for example, has a cosmopolitan distribution and plays a dominant role in wetland vegetation (Larridon et al., 2013). This family is represented by about 5,000 species distributed among 104-122 genera (Jung; Choi, 2013). Some representative species of the genus *Fimbristylis* have been recognized for their allelopathic potential (Ismail; Siddique, 2012a; Islam; Kato-Noguchi, 2016). According to Siddique and

Ismail (2013), *Fimbristylis miliacea* (L.) Vahl is a species with a phytotoxic potential that exerts negative physiological effects on rice crops. Some allelochemicals have been isolated from this species (Ismail; Siddique, 2012b).

Despite the allelopathic tests carried out in rice crops, there are no reports in the literature describing the phytotoxic effects of *F. miliacea* extracts on the germination of seeds of other plant species. Thus, bearing in mind that lettuce (*Lactuca sativa* L.) has been widely used in assays for the identification and determination of allelopathic effects, as it presents sensitivity to some classes of secondary metabolites (Ferreira and Aquila, 2000; Passos et al. , 2014), this study aims to analyze the phytochemical profile and the allelopathic effects of aqueous extracts of *Fimbristylis miliacea* (L.) Vahl on the germination of seeds of *Lactuca sativa* L. (lettuce) and *Emilia fosbergii* Nicolson by performing laboratory bioassays.

## 2. Material and Methods

### *2.1 Plant Material*

*Fimbristylis miliacea* (L.) Vahl was collected at the Igreja Nova, Alagoas, Brazil in July/2016 to July/2017. The species was identified by the specialist Dr. Ana Paula do Nascimento Prata, and subsequently an *exsiccate* was deposited at the Herbarium of the Environment Institute of Alagoas under no. MAC-63548.

### *2.2 Obtaining Extracts*

Shoots and roots of *F. miliacea* were dried in an oven with forced air circulation at 45ºC, and pulverized using a knife mill. The obtained powder was stored in plastic bags at -20ºC until the moment of use for the preparation of the aqueous extract.

To obtain the aqueous extract, *F. miliacea* shoot and root powders were separately used, and a 30-g 100 $mL^{-1}$ (30%) aqueous stock solution was prepared (weight/volume). This solution remained at rest for 24 hours. The extracts obtained were filtered and used for dilutions at the concentrations of 0.94%, 1.87%, 3.75%, 7.5%, 15%, and 30% (adapted from Pereira et al., 2018).

### *2.3 Evaluation of the Allelopathic Effect*

The aqueous extracts of *F. miliacea* (shoots and roots) were used on *Lactuca sativa* L. and *Emilia fosbergii* Nicolson seeds by controlling the germination of seeds at seven different concentrations (0, 0.94, 1.87, 3.75, 7.5, 15, and 30%). We used transparent plastic boxes with lids (11x11x4 cm) previously disinfected with alcohol and lined with two previously autoclaved Germitest paper sheets at 120ºC, proper relative humidity, proper temperature (25ºC and photoperiod of 12 hours), and luminosity artificially controlled in BOD (biochemical oxygen demand) germination chambers. Each plate received 30 lettuce seeds (*Lactuca sativa* L.) (local trade) and 50 *Emilia* seeds (*Emilia fosbergii* Nicolson) (collected in the study area) moistened with 6 mL of the aqueous extracts. As a negative control, 6 mL of distilled water were used. The experiment was conducted in triplicate. The evaluation was performed daily for seven days for lettuce and 14 days for Emilia. The radicle emission was the criterion for seed germination evaluation.

### *2.4 Phytochemical Screening*

The phytochemical screening was based on the methodology proposed by Matos (1997). From the sample of aqueous extracts obtained from *F. miliacea*, 35.0 mL were separated for phytochemical prospecting divided into seven 3.0 mL portions in test tubes numbered from "1" to "7." Qualitative and semi-quantitative tests were performed for phenols and tannins (by reaction with ferric chloride), anthocyanins, catechins and flavonoids (by pH variation test with sodium hydroxide and hydrochloric acid), saponins (foam test), alkaloids (Dragendorff identification), and terpenoids.

### *2.5 High Performance Liquid Chromatography (HPLC)*

For the separation and analysis of chemical compounds, the high performance liquid chromatography (HPLC) was used as described by Bezerra et al. (2019). 1 mL of the aqueous extract of the shoot of *F. miliacea* was filtered through a 0.20 µm milipore filter using a syringe and transferred to appropriate vials for HPLC analysis. The chromatographic profile was performed on HPLC with ultraviolet detector (UV) and diode array (DAD), where the extracts were injected at a flow rate of 0.6 mL/min for 72 minutes using one phase column per stationary phase (Jupiter 5u reverse column C18 300A and a mixture of methanol, water and 0.1% trifluoroacetic acid per mobile phase). Reference standards were used to identify possible chemical compounds (Table 1). Chromatograms were recorded at the wavelengths 254, 275 and 320 nm.

Table 1. Standard used for identification of chemicals compounds by high performance liquid chromatography

| Retention time (min) | Compounds | λ1 (nm) | λ2 (nm) | λ3 (nm) |
|---|---|---|---|---|
| 11,17 | Gallic acid | 195 | 218 | 283 |
| 27,41 | Chlorogenic acid | 250 | 288 | 315 |
| 33,62 | Epi-guaipyridine | - | 276 | 315 |
| 34,00 | Guaipyridine | - | 276 | 315 |
| 50,68 | Luteolin | | 259 | 332 |
| 52,12 | Apigenin | 256 | 268 | 317 |
| 54,32 | Chrysin | - | 271 | - |
| 55,78 | Acacetin | 272 | 320 | 375 |

*2.6 Statistical Analyses*

The analyzed variables were germination percentage and germination speed index. The germination speed index (GSI) was calculated according to the Maguire equation (1962):

GSI = (N1/E1) + (N2/E2) + ... + (Nn/En)

Where: GSI = germination speed index; E1, E2, En = number of seedlings in the first, second and last counts. N1, N2, Nn = number of days of sowing to the first, second and last count.

The data were submitted to analysis of variance at 5 and 1% of probability by the F-test, and after the analysis of variance and a significant extract effect, data were adjusted according to the non-linear regression model, log-logistic type, with three parameters proposed by Seefeldt et al. (1995) to determine the CL50 (inhibitory concentration equivalent to 50% effect in relation to the control), using the Sigmaplot software:

$$y = \frac{a}{\left[1 + \left(\frac{x}{b}\right)^{c}\right]}$$

where: y = percentage control; x = extract concentration; *a*, *b*, and *c*, estimated parameters of the equation, so that *a* = amplitude between the maximum point and the minimum point of the variable, *b* = concentration that provides 50% response of the variable and *c* = slope of the curve around *b*.

$CL_{50}$ is the concentration of the extract in % that provides the value of 50% control or reduction of growth of the recipient species. The values of the control were considered as 100%, and the results of each variable of the other treatments were calculated in relation to the control using the following formula:

RV (%) = 100*V/Tm

Where, RV = variable in relation to the control (%); V = analyzed variable (G, GSI); Tm = mean of the control (%).

## 3. Results and Discussion

*3.1 Allelopathic Activity*

The aqueous extracts from the aerial part (AP) and the underground part (UP) of *F. miliace* did not show any significant difference as to the variable germination (G). The extracts did not inhibited the germination of *Lactuca sativa* seeds in concentrations analyzed (0.94, 1.87, 3.75, 7.5, 15, and 30%) (Figure 1A). As for the variable germination speed index (GSI), the extract from the underground part had an allelopathic effect less than that of the aerial part (Figure 1B).

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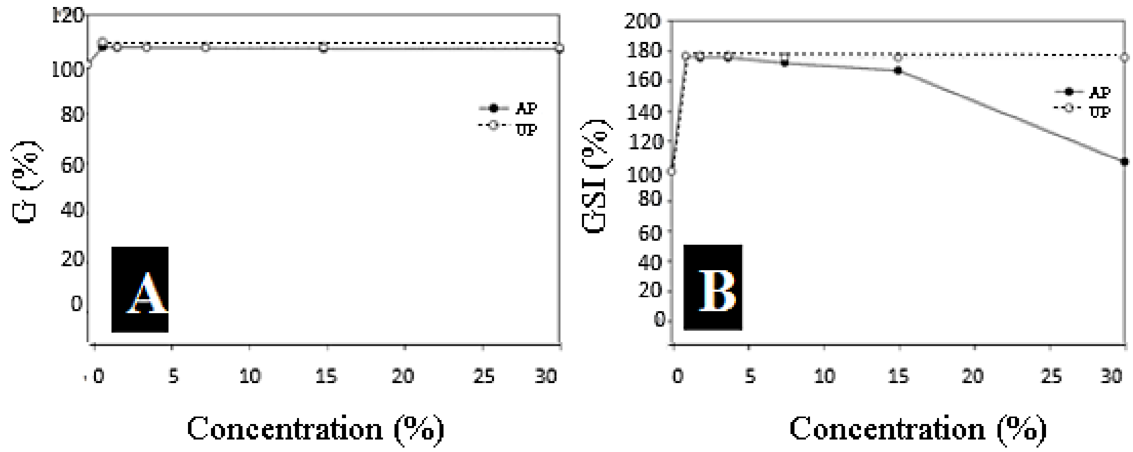


Figure 1A and 1B. Germination percentage (G) and germination speed index (GSI) of Lactuca sativa L. as a function of treatment (AP and UP) and increasing concentrations of the aqueous extract of *Fimbristylis miliacea* (L.) Vahl

Unlike the effect caused on lettuce seeds, the aqueous extracts of *F. miliacea* (aerial and underground) show significant allelopathic action when evaluated on seeds of the recipient species *Emilia fosbergii* (Emilia). There was no difference between the extracts tested as to the variables G and GSI (Figure 2A and 2B). This suggests that *E. fosbergii* is a species susceptible to the allelochemicals of *F. miliacea*.

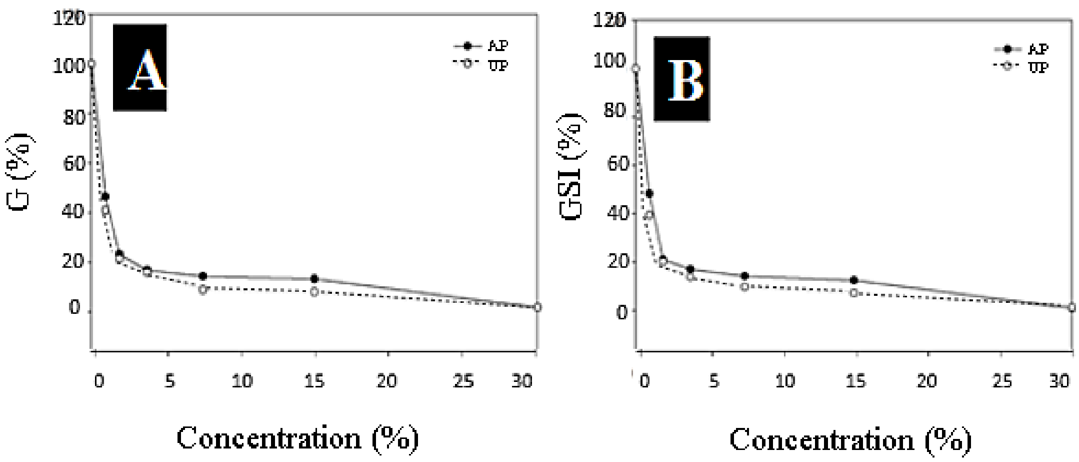


Figure 2A and 2B. Germination percentage (G) and germination speed index (GSI) of *Emilia fosbergii* Nicolson as a function of treatment (AP and UP) and increasing concentrations of the aqueous extract of *Fimbristylis miliacea* (L.) Vahl

In a research carried out by Ismail and Siddique (2012a), the aqueous extracts of the stem of *F. miliacea* significantly reduced the root growth of rice plants with the increase in concentration levels (12.5 g/L, 25 g/L, and 50 g/L). Regarding physiological and biochemical effects of the aqueous extract of *F. miliacea*, it was proven that this weed contributes to the inhibition of melondialdehyde contents and impairs the activity of the antioxidant enzymes known as catalase and peroxidase of the tested rice varieties (Siddique; Ismail, 2013). The aqueous extracts of another species of the genus *Fimbristylis* (*Fimbristylis dichotoma* L.) also had an allelopathic effect when evaluated on the development of lettuce (Islam; Kato-Noguchi, 2016).

*3.2 Phytochemical Screening*

The chemical reactions help to elucidate the classes of secondary metabolites present in the aqueous extract of *F. miliacea*. They revealed the presence of flobafenic tannins, catechins, flavanones, flavones, flavonols, xanthons, flavononols, triterpenes, and alkaloids. It is suggested that there is no presence of chemical compounds such as anthocyanin, anthocyanidin, leucoanthocyanidin and saponins in the analyzed aqueous extract (Table 2).

Table 2: Phytochemical screening of aqueous extract of aerial and underground parts of *Fimbristylis miliacea* (L.) Vahl

| Test | Aerial part | Underground part |
|---|---|---|
| Phenols | - | - |
| Tannins | - | - |
| Tannins flobafenics | - | + |
| Anthocyanin | - | - |
| Anthocyanidin | - | - |
| Leucoanthocyanidin | - | - |
| Catechins | + | + |
| Flavanones | - | ++ |
| Flavones | ++ | ++ |
| Flavonols and xanthons | ++ | ++ |
| Chalcones and aurones | - | - |
| Flavononols | - | + |
| Steroids | - | - |
| Triterpenoid | +++ | ++ |
| Saponins | - | - |
| Alkaloids | ++ | ++ |

Studies that show the chemical composition of *F. miliacea* are scarce in the literature, which hinders a comprehensive discussion about the possible phytochemicals involved in the allelopathic effects attributed to this species. However, it is important to note that some substances belonging to the three classes of secondary metabolites (phenolic compounds, terpenes and alkaloids) have phytotoxic effects (Latif et al., 2017).

### *3.3 High Performance Liquid Chromatography (HPLC)*

Through the techniques used in high performance liquid chromatography to identify the chemical compounds present in the aqueous extract of the aerial part of *F. miliacea*, it was possible to verify the occurrence of phenolic compounds, namely gallic acid (one of the major substances in the extract), chlorogenic acid, rutin, luteolin, apigenin, and acacetin (Figure 3A). Two alkaloids known as epi-guaipyridine and guaipyridine were also identified through the chromatographic run of the aqueous extract of *F. miliacea* (Figure 3B).

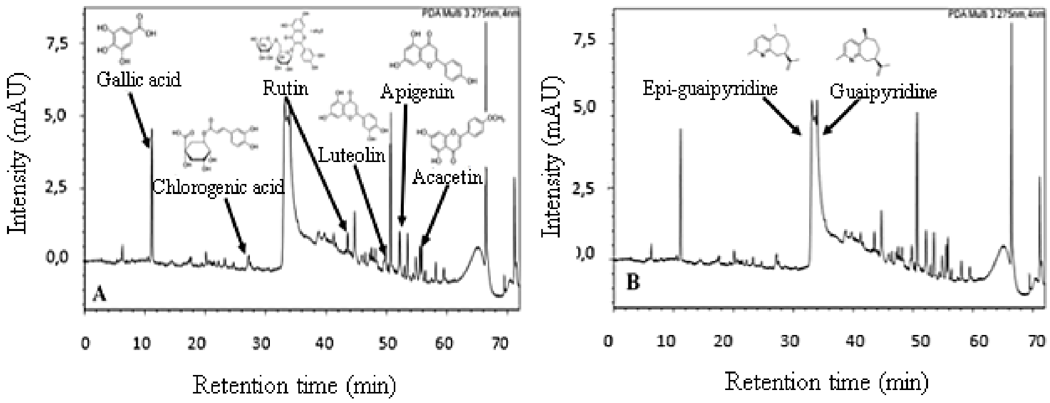


Figure 3A. Phenolic compounds identified in the chromatogram of the aqueous extract of *Fimbristylis miliacea* (L.) Vahl (aerial part) at a wavelength of 275nm; Figure 3B. Alkaloids identified in the same sample

The phenolic compounds identified by HPLC in this research can be directly associated with the allelopathic effects of the aqueous extract of *F. miliacea* on the germination of lettuce seeds. Potentially allelopathic phenolic compounds were identified in extracts of another species of the Cyperaceae family (*Cyperus rotundus* L.) using HPLC. Alsaadawi and Salih (2009) reported the presence of ferulic, caffeic, hydroxylbenzoic, syringe, chlorogenic and p-coumaric acids in the sprouts of this species. According to Li et al. (2010), phenolic allelochemicals can lead to increased permeability of the cell membrane. Consequently, the cell contents are spilled and there is an increase in lipid peroxidation. Finally, there is slow growth or death of plant tissue.

No relations was found between the allelopathic effect and the substances epi-guaipyridine and guaipyridine identified in the sample of the aqueous extract of *F. miliacea* by HPLC. However, other types of alkaloids are cited by some authors as phytotoxic substances even during the development of lettuce (Bravo et al., 2010; Sasamoto et al., 2015).

## 4. Conclusion

The aqueous extract of *F. miliacea* presented important chemical constituents (gallic acid, chlorogenic acid, rutin, luteolin, apigenin, acacetin, and alkaloids) that can be directly related to the allelopathic effects observed in laboratory bioassays. *E. fosbergii* seeds were more susceptible to the allelochemicals present in the aqueous extracts of *F. miliacea* compared to seeds of *L. sativa,* which germinated normally. It is important that further research be developed in order to isolate the main allelochemicals of *F. miliacea* and to carry out new phytotoxic bioassays.

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