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TRADITIONAL MEDICINE Research Article ISSN: 2766-9963

The Antifungal Activity and Phytochemical Screening of a Traditional South American Remedy: *Kyllinga vaginata* Against *Fusarium graminearum*

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Received : January 14, 2021
Published : March 12, 2021

ABSTRACT

Kyllinga vaginata Lam. a native species of Paraguay, which is popularly known as "kapiikati", is marketed by Spanish hebalists known as "yuyeras". *K. vaginata* Lam. has many pharmacologic effects such as diuretic, antispasmodic, diaphoretic, also used for the treatment of fungal diseases like leucorrhea. Its secondary metabolites are useful array of natural products with remarkable biological activities in medicinal components, food additives therapies, aromatic, and culinary purposes. In the current research phytochemical experiments revealed that the presence of several natural products in the rhizome of *K. vaginata* (ethanol crude extract), such as flavonoids, phenolic compounds and lipids. With the evidence of traditional anti-leucorrhea uses, the present study also investigated to explore hidden alternative antifungal activity of *K. vaginata*. As a result of this translational approach, microorganism inhibition growth tests were performed on these crude EtOH extracts and revealed antifungal activity against *Fusarium graminearum*, a phytopathogen of wheat. Useful identification features are cited for a fast/economic quality control of the rhizome of *K. vaginata*, the presence of secondary metabolites are declared and main chemical families of biologically active compounds are proposed for the first time for *K. vaginata*. Finally antioxidant activity of these roots were studied and the inhibitory activity against *F. graminearum*, proposed by this translational approach.

KEYWORDS: Cyperaceae; *Kyllinga vaginata*; kapiikati; Paraguay herbal medicine; Yuyeras; Fusarium *graminearum*; Phytopathogen

ISSN: 2766-9963

INTRODUCTION

From many years, research on the plants is continuing for its beneficial and medicinal values in treatment of various diseases. Plants are also worshipped in some religions and perform rituals to it. It is a known fact that the research on extracting, processing, and isolating bioactive molecules from plants is very expensive procedure. Yet, it is more efficient, worth to work on identifying medicinal properties to use as a traditional medicine (ethnopharmacology and ethnobotany) for human benefit [1,2].

Like many countries, Paraguay in Latin America, has a strong tradition of using medicinal plants for primary health [3]. It is evident that the Paraguay is one of the countries that consume the most tea-based herbal medicines worldwide [4,5].

Locally, at most of the street plant markets of Paraguay, we find the mixture of species popularly known as "kapiikati", which in the Guarani language means "herb with a strong smell" and marketed by the "yuyeras". "kapiikati ", in fact includes four different species of the genus Kyllinga and another species as *Scleria distans*. The main species used is *Kyllinga vaginata* Lam. (Family: Cyperaceae) which is widely distributed in the country and of which only the rhizome is used in "mate" and "tereré". Such species is cataloged as a substitute for *K. odorata* Vahl., validated as medicinal plant and used in the treatment of leucorrhoea [6].

This work focuses on the chemical and biological study of *Kyllinga vaginata* Lam., the main component of the herbal mixture named "kapiikati" whose rhizomes are consumed in "mate" or "terere" for their digestive, diuretic, sedative, tonic, antispasmodic properties, and vaginal disorders [6].

Apart from this traditional herbal component, Paraguay is also a world exporter of wheat. Fusarium head blight is one of the most important fungal disease of cereals caused by the fungus *Fusarium graminearum*. This pathogen can therefore lead not only to direct crop losses, but also to the production of mycotoxins, toxic secondary metabolites for humans and animals that ingest contaminated food [7].

Based on the traditional medicine information about problems related with diseases caused by fungal pathogens (as for example leucorrhoea) and the high number of antifungal natural products [8], we proceed to explore the antifungal potential of *K. vaginata* applied to the fight against the fungal wheat diseases triggered by Fusarium.

MATERIAL AND METHODS

Plant Material

Roots of *Kyllinga vaginata* (Figure 1) were collected in Paraguay near Ypacarai, at the Central Department (25°22´59.88´´S-57°16´0.12´´O) and identified by B. Benitez (Department of Botany, National University of Asuncion, Paraguay). An analysis of the taxonomic identity of the *K. vaginata* is made by means of identification keys, and worldwide reference database including morphological, anatomical and histochemical descriptions contrasted with the literature and compared with international herbarium material. Three voucher specimens (M.E.Blanc 1, 28-I-2016, M.E.Blanc 2, 28-I-2016 and M.E.Blanc 3, 28-I-2016) have been deposited at the "Herbarium FACEN" of the Biology Department at the National University of Asunción (UNA), San Lorenzo, Paraguay.

Figure 1: Roots of *Kyllinga vaginata*.

Extract Preparation

Drying and cutting of the rhizome: The collected plant specimen, was cleaned and dried at room temperature, protected from light and moisture [9]. The part of the plant used in the present study was the rhizome, being the part used in folk medicine from Paraguay [10].

Grinding: The rhizomes were first cut with scissors and grounded with a screw mill to make a powder form. Finally, a total of 6,505 kg of rhizomes´ powder was obtained.

Eco-extraction: 6,0 kg of dried and powdered rhizomes were extracted by maceration with approximately 50 liters of ethanol 96° (EtOH) for a period of 30 days, with agitation of at least three times per week. Two more eco-extractions were repeated (for shorter period of 15 days for each extraction). Finally, the crude EtOH extract ("KYC") was filtered by gravity and subsequently evaporation of the solvent was carried out under reduced pressure in a rotary evaporator. The yield of the eco-extraction was approximately 5,88% (353g of crude extract). The extract was stored at 4°C.

DOI: https://doi.org/10.35702/Trad.10006

ISSN: 2766-9963

Phytochemical Analysis

Phytochemical screening: Several procedures used for the detection of the main families of natural products were used. Their respective methodologies and references are listed in table 1.

Quantification of total phenols content: The phenolic content was quantified by the colorimetric method of Folin-Ciocalteu (each sample was analyzed in triplicate [11]). The methanol solutions of the crude extract were prepared at a 1.22 $mg.mL^{-1}$ final concentration; the solution was placed in a 10 mL flask, then 2 mL of distilled water were added and the mixture was actively mixed. 200 µL of Folin-Ciocalteu reagent were added and incubated during 5 minutes before addition of 1.5mL of a 20% sodium carbonate (Na_2CO_3) solution. Final volume was adjusted with distilled water to 10 mL. The reaction mixture was mixed and allowed to react during 60 minutes. After 60 min., the samples were analyzed on a visible/UV spectrophotometer at a wavelength of 760 nm. A standard curve with gallic acid was performed and used to estimate phenolic content of each sample.

Quantification of total flavonoids content: The method used was described by Woisky and Salatino [12]. The methanolic solutions of the ethanol extracts were prepared with a final concentration of 1,024 $mg.mL^{-1}$. In each reaction tube, 2 mL of sample solution and 2 mL of 2% aluminum chloride solution ($AlCl_3$) were mixed and absorbance at 420 nm were measured in a visible/UV spectrophotometer. Each measure was made in triplicate. A standard curve was performed with quercetin standard solutions to estimate flavonoids content of each sample.

Antioxidant Activity

This test was performed as described by Budhiyanti and collaborators [13]. Methanol solution of the ethanol extracts was prepared at a final concentration of 0.45 $mg.mL^{-1}$. Then 100 µL of each sample to be tested were mixed with 3.9 mL of a 2,2–diphenyl-1-picrylhydrazyl (DPPH) with a concentration of 0.1 mM. All samples were allowed to stand at room temperature for 1 hour. Subsequently, absorbance at 517 nm were measured with a visible/UV spectrophotometer for triplicate samples. A standard curve with ascorbic acid was performed and the formula to quantify antioxidant activity in the sample was used as described by Skoog and coworkers [14].

Antifungal Activity

This test was carried out following the guidelines of Ochoa Fuentes and coworkers [15] with modifications. The antifungal activity of the crude extract KYC was determined, through the percentage of growth inhibition, of the filamentous fungi: *Fusarium graminearum* that was provided by Dr. Andrea Arrúa of the Multidisciplinary Center for Technological Research (CEMIT-UNA).

Sample preparation: The suspensions of the crude extract were prepared as follows: 0.28g and 0.56g of the "KYC" were weighed to obtain final concentrations of 2000 ppm and 4000 ppm respectively. 5 mL of absolute ethanol were added and the mixtures were sonicated for 20 min. Then 65 mL of sterile water and 70 mL of PDA culture medium were added to this mixture at 50°C.

Procedure: The filamentous fungus to be evaluated were removed with a punch and placed in the center of the plates, they were incubated at 25 ± 2°C for 6 days and finally the fungus growth rates were measured. The negative control consisted of incubating the fungus in plates with medium without extract. The tests were carried out in triplicate.

The percentage of inhibition was calculated according to Tequida and colleagues [16], taking into account the following equation:

% Growth: growth diameter of fungi in extract x 100 / Negative control diameter

RESULTS

Crude Extract Phytochemical Characterization

Results of the phytochemical compounds screening confirmed the presence of several families of natural products: alkaloids, tannins, flavonoids, triterpenes, steroids, leucoanthocyanidins and saponins in the tested extracts. It is important to note that quinones have not been detected under these test conditions and the result related with the presence of coumarins remains inconclusive (Table 1).

DOI: https://doi.org/10.35702/Trad.10006

ISSN: 2766-9963

Secondary Metabolites Family	Phytochemical tests	Result*	Reference
Alkaloids	Mayer	-	(Iqbal E, et al. 2015) [17]
Tanins	$FeCl_3$	+ (green)	(Ugochukwu SC, et al. 2013) [18]
	Gelly-Salt	+	(Samantha T, et al. 2012) [19]
Flavonoids	$AlCl_3$10%	+ + +	(Aguelo I, et al. 2013) [20]
	Shinoda	+ + +	(Chew TL, et al. 2011) [21]
Triterpenes/ steroids	Salkowsky	+	(Iqbal E, et al. 2015) [17]
Quinones	Borntrager	-	(Khandelwal K, et al. 2008) [22]
Coumarins	Baljet	-	(Nabi N, et al. 2017) [23]
	Fluorescence of coumarins	+	(Poumale HM, et al. 2013) [24]
Leucoanthocyanidins	Rosenheim	+	(Bonilla-Ríos MC, et al. 2014) [25]
Saponins	Afrosymmetric	+ + +	(Bonilla-Ríos MC, et al. 2014) [25]

Table 1: Qualitative results of the phytochemical profile performed on the ethanol extracts. *(+)-faint coloration, (++)-medium coloration, (+++)-intense coloration, (-)-absence of coloration.

From ETOH extract the quantification of flavonoids and total phenols was carried out, measuring the absorbance at 760 nm. Presenting a concentration of total phenols about 1.22 mg/mL-1. Another measure at 420 nm was done detecting a concentration of flavonoids about 1,024 mg/mL^{-1}.

Table 2 shows the test results for the total phenols and flavonoids content in the samples calculated and an estimation of the gallic acid and quercetine equivalent content (mg/g^{-1}).

Extract	Absorbance 760 nm	Concentration (mg/mL-1)	Equivalents of Gallic acid (mg/g-1)
KYC	0,442	1,22	0,5 ± 0,14
	Absorbance 420 nm	Concentration (mg/mL-1)	Equivalents of quercetine (mg/g-1)
KYC	0,075	1,024	Lower than the quantification limit

Table 2: Test result for total phenols (Abs 760 nm) and flavonoids (Abs 420 nm) content.

Antioxidant Activity

The antioxidant activity of the EtOH extracts is found by means of the reaction with DPPH. Table 3 shows the test results for the antioxidant activity tests. The rate of inhibition of DDPH reaction of EtOH extract was 36,4%.

Extract	Absorbance at 517 nm	Inhibition (%)
Negative control	0,637	--
Green tea (Positive control)	0,182	71,414
KYC	0,405	36,4

Table 3: Antioxidant activity.

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ISSN: 2766-9963

Antifungal Activity

Inhibitory activity against *Fusarium graminearum* 150 was obtained at the two test concentrations, 2000 ppm and 4000 ppm, with a growth inhibition percentage of 72.9 ± 0.53 and 82.5 ± 0.14 respectively.

DISCUSSION

The present article offers an exploration of the phytochemical natural products present in *Kyllinga vaginata*. The study allows identifying the presence of several secondary families of metabolites as flavonoids, phenolic compounds, fats or lipids. Moreover, it has been shown that the crude extracts of *Kyllinga vaginata* have antifungal potency against *Fusarium graminearum* and interesting antioxidant properties.

At this study we show that *K. vaginata* could be considered as a valuable medicinal plant with an interesting antifungal but also antioxidant properties.

The presented article illustrates the interest of ethnopharmacological information (traditional anti-leucorrhoea applications of *K. vaginata*) as a source of information for different complementary applications, as for example the inhibition of growth of *F. graminearum*. As result of this translational research, the ethnopharmacological information about the treatment of diseases for humans can be considered a source of innovative treatments for the plant diseases.

AKNOWLEDGEMENTS

The author would like to thank Dr. Andrea Arrua (CEMIT, UNA) for the kind donation of *Fusarium graminearum*. The authors would like to acknowledge for financial support to the project "Jardin Créole Médicinal" from "Région Guadeloupe" and "Communauté d'agglomération du Sud Basse-Terre (CASBT)".

CONFLICTS OF INTEREST

The authors have indicated that they have no other conflicts of interest regarding the content of this article.

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Citation: Blanc RME, et al. (2021). The Antifungal Activity and Phytochemical Screening of a Traditional South American Remedy: Kyllinga vaginata Against Fusarium graminearum. Traditional Medicine. 2(1):06.

DOI: https://doi.org/10.35702/Trad.10006