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In vitro assay of *Bidens pilosa* Linn. aqueous extract against postharvest fungal pathogens on Corn and Peanut

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Abstract

A bioassay was conducted to assess the antifungal effects of the different concentrations of *Biden pilosa* Linn. aqueous extract against fungal pathogens isolated from corn and peanut seeds. The assay employed the disk diffusion technique to determine the effects of the diffusible metabolites from *B. pilosa* on the growth of the fungal species on potato dextrose agar (PDA). The aqueous extract showed significant activity against *Aspergillus flavus, A. niger, Fusarium* sp., and *Penicillium* sp. from corn seeds. The best antifungal activity was observed in *A. niger* with inhibitory zones as wide as 19.72mm in diameter. On the other hand, the fungal isolates from peanut namely, *A. flavus, A. niger, Penicillium* sp., and *Rhizopus stolonifer* showed sensitivity to the aqueous extract from *B. pilosa*. The best antifungal activity was recorded in *Penicillium* sp. with the widest zone of inhibition of 24.87mm at 24 hours after incubation (HAI). This *in vitro* study, therefore, confirms that the *B. pilosa* aqueous extract inhibits the growth of fungal species associated with corn and peanut seeds.

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Introduction

Corn and peanut seeds are vulnerable to pathogenic fungal species before and after harvesting. Their association with the stored seeds may eventually result in the deterioration of seed quality. Although stored seeds apparently look healthy because of the absence of physical damage, however, these may be contaminated with high levels of mycotoxins produced by certain species of fungal pathogens. Mycotoxins are fungal metabolites that cause grain quality deterioration, poor germination potential, and reduced vigor. To prevent these problems, different control strategies should be employed such as irradiation, chemical treatment, and biological control. However, irradiation of seeds before storage is costly while the application of chemical treatments poses hazards to humans and animals. With these issues at hand, one of the promising options is the application of botanical pesticides.

Several studies had been conducted on the use of weed extract to control the growth of plant pathogenic organisms. The water extracts from the weed species (*A. conyzoides, Oxalis corniculata, Phyllanthus debilis, Vernonia cinerea,* and *Desmodium trifolium*) were assayed for their antifungal activity against some plant pathogenic fungi (Iqbal *et al*., 2001).

The extract from *A. conyzoides* inhibited the mycelial growth of *Rhizoctonia solani, Aspergillus niger,* and *Phomopsis theae*. In another study, the extract from *O. corniculata* was active against *A. niger* while *P. debilis* suppressed the growth of *P. theae*. The activity generally declined after three days of incubation, while *A. conyzoides* remained active for nine days after incubation. Ethanolic extract of *Datura stramonium* also contains significant antifungal potential against some important plant pathogenic fungi and thus, could be used as an alternative to chemical fungicides for the management of fungal infection in plants (Sharma *et al*., 2014).

There were previous studies on *Bidens pilosa*, a common weed species in the tropics, focusing on its antibacterial effects against human pathogens. Silva *et al*. (2014) evaluated nine extracts from *B. pilosa* (root, stem, flower, and leaves) and *Annona crassiflora* (rind fruit, stem, leaves, seed, and pulp) against 60 oxacillin resistant *Staphylococcus aureus* (ORSA) and *S. aureus* ATCC6538. They found that extracts from the leaves of *B. pilosa* had significantly wider inhibition zone diameters than chlorexidine against ORSA, and the extracts were more active against *S. aureus* ATCC. The presence of variable alkaloids, flavonoids, tannins, and saponins was observed which may be responsible for its antibacterial activities.

The antifungal properties of *B. pilosa* were documented in some studies involving plant pathogenic fungal species. Deba *et al*. (2007) first evaluated the antifungal potential of this plant against *Corticium rolfsii, Fusarium solani, and Fusarium oxysporum* using the hot water extracts from the roots, stem, and leaves. They found that *C. rolfsii* was most suppressed as its growth was reduced almost all the tested doses followed *by F. oxysporum* and *F. solani*. Extracts from stems and roots exhibited greater fungicidal action than the extracts from the leaves. In another experiment, the team also demonstrated the antifungal effects of the essential oils and aqueous extracts from the flowers and leaves of *B. pilosa* using the three fungal species. They again concluded that the extracts and oils had antifungal activity on the fungal pathogens (Deba *et al*., 2008).

Polyacetylenes, polyacetylene glycosides, flavonoids, flavone glycosides, aurones, chalcones, okanin glycosides, phenolic acids, terpenes, pheophytins, fatty acids, and phytosterols are among the chemical ingredients identified or isolated from the various portions of *B. pilosa* (Xuan & Khanh, 2016). Many of these have been identified as bioactive chemicals with pharmacological potential.

According to Silva *et al.* (2011), as cited by Bartolome *et al*. (2013), 201 compounds have been identified from this plant as compiled previously, comprising of 70 aliphatics, 60 flavonoids, 25 terpenoids, 19 phenylpropanoids, 13 aromatics, 8 porphyrins, and 6 other compounds.

This study focused on the assay of *B. pilosa* aqueous extract against common fungal pathogens associated with corn and peanut seeds. The antifungal effect of the extract was determined under *in vitro* conditions.

Materials and methods

Fungal Isolates

The fungal species were isolated from corn and peanut seeds using potato dextrose agar (PDA) medium. These isolates were maintained in agar slants and reactivated on fresh medium prior to the bioassay.

Bidens pilosa Leaves

Fresh leaves of *B. pilosa* (Fig. 1) were collected within the Central Mindanao University campus. The leaf samples were thoroughly washed with tap water and then with distilled water.

Fig. 1. Morphology of *Bidens pilosa* Linn.

Experimental Design and Treatments

The experiment was laid out in a Complete Randomized Design (CRD) with three replications for each treatment. Five subsamples were provided per replicate. These were the treatments:

T_1 - Negative Control (Sterile distilled water)

T_2 - Positive Control (Daconil fungicide)

T_3 – Pure extract of *B. pilosa*

T_4 – 1ml *B. pilosa* extract : 1ml sterile distilled water (SDW)

T_5 – 1ml *B. pilosa* extract : 3ml sterile distilled water (SDW)

T_6 – 1ml *B. pilosa* extract : 5ml sterile distilled water (SDW)

T_7 – 1ml *B. pilosa* extract : 8ml sterile distilled water (SDW)

T_8 – 1ml *B. pilosa* extract : 10ml sterile distilled water (SDW)

Preparation of Aqueous Extract

The aqueous extract of *B. pilosa* was prepared by blending 100g of washed fresh leaves in 200ml of sterile distilled water (SDW). The mixture was filtered using a double layer of filter paper to remove debris and suspended materials in the mixture. The extract was placed in glass containers and stored at 4°C until further use. This served as the stock extract for the treatment application.

In-vitro Assay of Weed Extracts Against Fungal Pathogens

The antifungal activity of *B. pilosa* extract was evaluated by employing the disk diffusion technique. One millimeter of fungal suspension was placed at the center of a sterile Petri plate. A sterile PDA medium was poured on the plate and was rotated alternately clockwise, counter clockwise, and crosswise to allow even distribution of the fungal suspension. The mixture was allowed to congeal before the assay of the extract.

Based on the disk diffusion technique, sterile paper disks were soaked in the prepared treatments for 10 minutes. The soaked paper disks were aseptically transferred to the culture plate.

The plates were then incubated at room conditions. The area showing fungal growth inhibition was assessed using a ruler at 24, 48, and 72 hours after incubation (HAI) of the culture plates. The zone of inhibition was computed by getting the average of the crosswise and lengthwise zone of clearing around the paper disk previously soaked on the treatment.

Statistical Analysis

All data were subjected to analysis of variance (ANOVA) and the treatment mean comparison was done using Tukey's HSD test.

Results and discussion

Inhibitory Zones on the Growth of Fungal Species Isolated from Corn Seeds

The summary of the zones of inhibition on the growth of *Aspergillus flavus, A. niger*, *Fusarium* sp., and *Penicillium* sp. isolated from corn seeds is shown in Table 1. On *A. flavus* isolate, the inhibitory zone at 24 HAI reached as high as 8.17mm which was recorded from T_2 (Positive Control – Daconil). This was followed by T_3 (Pure *B. pilosa* extract) and T_4 (1ml *B. pilosa* extract : 1ml SDW) with statistically similar inhibitory zones of 5.87 and 4.98mm, respectively. Lower concentrations of *B. pilosa* had resulted in significantly smaller inhibitory zones with 2.41 and 2.77mm in T_7 (1ml *B. pilosa* extract : 8ml SDW) and T_8 (1ml *B. pilosa* extract : 10ml SDW), respectively. Although there was a significant reduction in the inhibition of *A. flavus* growth from 24 HAI to 48 HAI and 72 HAI, still the aqueous extracts from *B. pilosa* were found effective against the fungal pathogen, particularly in treatments with higher concentrations of this extract.

Table 1. Inhibitory zones (mm) on the growth of fungal species isolated from corn seeds and applied with different concentrations of *B. pilosa* aqueous extract at 24, 48, and 72 hours after incubation (HAI).

Treatment	*Aspergillus flavus*			*Aspergillus niger*			*Fusarium* sp.			*Penicillium* sp.		
	24 HAI	48 HAI	72 HAI	24 HAI	48 HAI	72 HAI	24 HAI	48 HAI	72 HAI	24 HAI	48 HAI	72 HAI
T_1-Negative Control (SDW)	0.00^{f}	0.00^{g}	0.00^{d}	0.00^{b}	0.00^{d}	0.00^{c}	0.00^{f}	0.00^{e}	0.00^{b}	0.00^{d}	0.00^{c}	0.00^{d}
T_2-Positive Control (Daconil)	8.17^{a}	2.71^{b}	1.22^{ab}	16.74^{a}	7.68^{a}	5.30^{ab}	3.06^{d}	2.04^{bc}	0.72^{ab}	1.09^{cd}	0.97^{bc}	0.97^{bcd}
T_3-Pure extract of *B. pilosa*	5.87^{b}	3.51^{a}	1.50^{a}	17.95^{a}	6.94^{ab}	6.35^{a}	4.75^{a}	2.64^{ab}	1.07^{ab}	12.64^{a}	4.23^{a}	4.93^{a}
T_4-1ml *B. pilosa* extract:1ml SDW	4.98^{bc}	2.52^{bc}	0.85^{abc}	19.72^{a}	6.40^{ab}	6.09^{a}	4.55^{ab}	2.98^{a}	1.24^{a}	4.05^{b}	1.42^{bc}	1.56^{bc}
T_5-1ml *B. pilosa* extract:3ml SDW	4.16^{cd}	1.92^{cd}	0.87^{abc}	15.49^{ab}	4.16^{abc}	2.73^{abc}	4.09^{bc}	2.14^{abc}	0.90^{ab}	5.51^{b}	2.49^{b}	2.49^{b}
T_6-1ml *B. pilosa* extract:5ml SDW	3.53^{cde}	1.48^{de}	0.49^{bcd}	15.61^{ab}	4.71^{abc}	3.52^{abc}	3.63^{cd}	1.56^{cd}	0.47^{ab}	2.67^{bcd}	1.22^{bc}	1.22^{bcd}
T_7-1ml *B. pilosa* extract:8ml SDW	2.41^{e}	0.95^{ef}	0.25^{cd}	8.98^{ab}	3.91^{bc}	1.93^{bc}	2.33^{e}	0.88^{de}	0.33^{ab}	1.69^{cd}	0.77^{c}	0.77^{cd}
T_8-1ml *B. pilosa* extract:10ml SDW	2.77^{de}	0.59^{fg}	0.08^{d}	6.75^{ab}	1.92^{cd}	1.42^{c}	2.22^{e}	0.63^{e}	0.37^{ab}	0.97^{cd}	0.35^{c}	0.17^{cd}
F-test	**	**	**	*	**	**	**	**	*	**	**	**

Means with the same letter in a column are not significantly different at a 5% level of probability based on Tukey's HSD test.

* - significant ** - highly significant

On *A. niger*, the inhibitory zones recorded at 24 HAI were statistically similar in treatment with Daconil fungicide (T_2) and all treatments with *B. pilosa* extract regardless of its concentration. This fungal pathogen showed sensitivity to the extract with inhibitory zones that range from 6.75 to 19.72mm. Still, the size of the inhibitory zones was reduced at 48 to 72 HAI across all treatments. However, the effectiveness of *B. pilosa* using pure extract (T_3) and those with concentrations of 1ml *B. pilosa* extract : 1ml SDW (T_4), 1ml *B. pilosa* extract : 3ml SDW (T_5) and, 1ml *B. pilosa* extract : 5ml SDW (T_6) was still comparable with the treatment using Daconil fungicide (T_2).

Fusarium sp. isolated from corn also showed sensitivity to *B. pilosa* extracts with inhibitory zones that vary significantly among treatments at 24 HAI. The widest zone of inhibition was recorded in T_3 (Pure extract of *B. pilosa*), T_4 (1ml *B. pilosa* extract: 1ml SDW), and T_5 (1ml *B. pilosa* extract: 3ml SDW) with 4.75, 4.55, and 4.09mm, respectively. On *Penicillium* sp., the treatment with pure *B. pilosa* extract (T_3) had exhibited the widest inhibitory zone of 12.64mm at 24 HAI. This was followed in descending order byT_5 (1ml *B. pilosa* extract : 3ml SDW), T_4 (1ml *B. pilosa* extract : 1ml SDW), and T_6 (1ml *B. pilosa* extract : 5ml SDW) with 5.51, 4.05, and 2.67mm, respectively. A similar trend was noted at 48 to 72 HAI although with a notable reduction of the inhibitory zones in all treatments. Based on the results, the four fungal pathogen isolates from corn were all sensitive to the aqueous extract from *B. pilosa* leaf samples. This herb has long been used in traditional medicine to treat various human ailments but there is limited information as regards the utilization of this plant as a biopesticide. As demonstrated in this study, *B. pilosa* has the potential to inhibit fungal pathogens associated with corn seeds. This conforms to the finding of Deba and his colleagues in 2007 when they first investigated the antifungal potential of this plant against *Corticium rolfsii, Fusarium solani,* and *Fusarium oxysporum* using the hot water extracts from the roots, stem, and leaves. Among the three fungal species, *C. rolfsii* was most suppressed as its growth was reduced in almost all the tested doses followed *by F. oxysporum* and *F. solani*. In another experiment, the essential oils and aqueous extracts from the flowers and leaves of *B. pilosa* were also found effective against the three fungal species (Deba *et al.,* 2008). In addition, the study of Ashafa and Afolayan (2009) revealed that the acetone, methanol, and water extracts of *B. pilosa* had also shown inhibitory effects on *Aspergillus niger, A. flavus,* and *Penicillium notatum* using the agar dilution method. The antifungal activities of *B. pilosa* may be attributed to its bioactive components comprising of 70 aliphatics, 60 flavonoids, 25 terpenoids, 19 phenylpropanoids, 13 aromatics, 8 porphyrins, and 6 other compounds (Silva *et al.*, 2011 as cited by Bartolome *et al*., 2013).

Inhibitory Zones on the Growth of Fungal Species Isolated from Peanut Seeds

The summary of the zones of inhibition on the growth of *Aspergillus flavus, A. niger*, *Penicillium* sp., and *Rhizopus stolonifer* isolated from peanut seeds is shown in Table 3 and Fig. 6. The assessment was done at 24, 48, and 72 hours after incubation (HAI).

Table 2. Inhibitory zones (mm) on the growth of fungal species isolated from peanut seeds and applied with different concentrations of *B. pilosa* aqueous extract at 24, 48, and 72 hours after incubation (HAI).

Treatment	*Aspergillus flavus*			*Aspergillus niger*			*Penicillium* sp.			*Rhizopus stolonifer.*		
	24 HAI	48 HAI	72 HAI	24 HAI	48 HAI	72 HAI	24 HAI	48 HAI	72 HAI	24 HAI	48 HAI	72 HAI
T_1-Negative Control (SDW)	0.00^{e}	0.00^{b}	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{b}	0.00^{e}	0.00^{d}	0.00^{d}	0.00^{c}	0.00^{d}	0.00^{b}
T_2-Positive Control (Daconil)	10.39^{a}	3.57^{a}	2.89^{a}	9.70^{a}	1.00^{a}	0.56^{a}	17.17^{bc}	12.27^{b}	9.97^{b}	0.03^{c}	0.00^{d}	0.00^{b}
T_3-Pure extract of *B. pilosa*	2.18^{b}	0.70^{b}	0.30^{bc}	2.95^{b}	0.45^{b}	0.25^{ab}	24.87^{a}	18.87^{a}	14.40^{a}	3.03^{a}	2.17^{a}	1.22^{a}
T_4-1ml *B. pilosa* extract:1ml SDW	1.97^{bc}	0.73^{b}	0.37^{bc}	3.31^{b}	0.43^{b}	0.15^{b}	22.93^{a}	16.65^{a}	13.07^{a}	2.13^{ab}	1.32^{abc}	0.79^{ab}
T_5-1ml *B. pilosa* extract:3ml SDW	1.68^{bc}	0.55^{b}	0.20^{bc}	1.85^{bc}	0.00^{c}	0.00^{b}	19.53^{b}	11.75^{b}	10.19^{b}	2.72^{a}	1.78^{ab}	0.77^{ab}
T_6-1ml *B. pilosa* extract:5ml SDW	1.32^{cd}	0.57^{b}	0.38^{b}	1.92^{bc}	0.25^{bc}	0.13^{b}	16.05^{c}	11.45^{b}	10.22^{b}	1.00^{bc}	0.68^{bcd}	0.28^{ab}
T_7-1ml *B. pilosa* extract:8ml SDW	0.65^{de}	0.30^{b}	0.15^{bc}	1.49^{bc}	0.07^{c}	0.05	13.14^{d}	9.82^{bc}	8.37^{bc}	0.75^{c}	0.33^{cd}	0.20^{ab}
T_8-1ml *B. pilosa* extract:10ml SDW	0.55^{e}	0.23^{b}	0.08^{bc}	1.42^{bc}	0.03^{c}	0.00^{b}	12.28^{d}	7.63^{c}	6.45^{c}	0.32^{c}	0.13^{cd}	0.10^{a}
F-test	**	**	**	**	**	**	**	**	**	**	**	**

Means with the same letter in a column are not significantly different at a 5% level of probability based on Tukey's HSD test.

** - highly significant

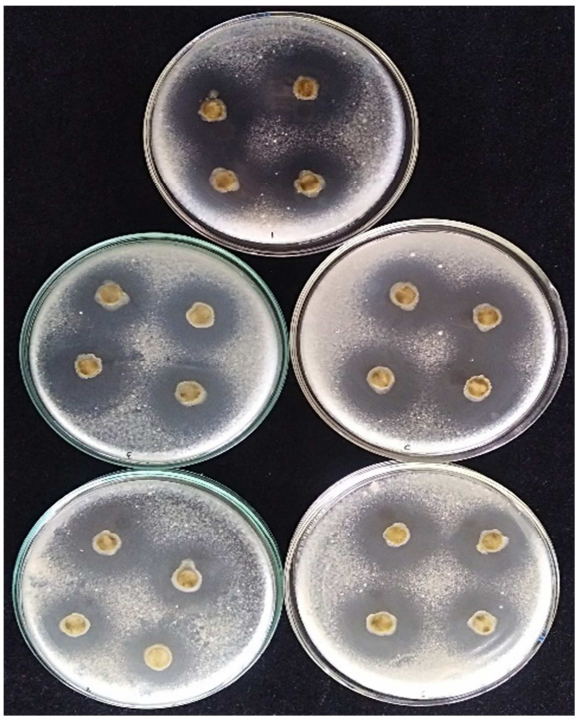

Fig. 2. Inhibitory zones on the growth of *Penicillium* sp. isolated from peanut and grown on potato dextrose agar (PDA) at 24 HAI.

The zone of inhibition on the growth of *A. flavus* at 24 HAI varied among treatments. The application of Daconil fungicide (T_2) had resulted in wider inhibitory zones of 10.39mm as compared to the treatments with *B. pilosa* extracts. On the treatments with aqueous extracts of the weed species, the widest inhibitory zone was observed in T_4 (1ml *B. pilosa* extract : 1ml SDW) and T_5 (1ml *B. pilosa* extract : 3ml SDW) with values comparable to T_3 (Pure extract of *B. pilosa*). The efficacy of the different treatments was still evident even at 48 and 72 HAI but the inhibitory zones were significantly reduced particularly in treatments with Daconil fungicide and with *B. pilosa* extracts.

A. niger had also shown sensitivity to the extracts with inhibitory zones that range from 1.42 to 3.39mm at 24 HAI. However, these values were significantly lower than the inhibitory zone on treatment with Daconil fungicide (T_2). A similar trend was again observed at 48 and 72 HAI wherein the degree of effectiveness of Daconil fungicide and the different concentrations of *B. pilosa* extracts had reduced to 1mm or even less than 1mm. Some treatments were even completely ineffective as early as 48 HAI (T_5-1ml *B. pilosa* extract : 3ml SDW).

The growth of *Penicillium* sp. was most suppressed on treatments with pure extract (T_3) and with 50% extract (T_4) at 24 to 72 HAI. The widest zone of inhibition was observed at 24 HAI with 24. 87 and 22.93mm in T_3 (Pure extract of *B. pilosa*) and T_4 (1ml *B. pilosa* extract: 1ml SDW), respectively. These values were significantly higher than the inhibitory zones in the positive control (T_2-Daconil) with 17.17mm. At 48 and 72 HAI, a similar trend was noted, with the reduction of the degree of effectiveness in treatments with Daconil fungicide and with varying concentrations of *B. pilosa* extracts.

On *R. stolonifer*, higher concentrations of *B. pilosa* extract were also effective in suppressing the growth of this fungal species at 24 to 48 HAI. The inhibitory zones on T_3 (Pure extract of *B. pilosa*), T_4 (1ml *B. pilosa* extract : 1ml SDW), and T_5 (1ml *B. pilosa* extract : 3ml SDW) were significantly higher compared with the inhibitory zone in the positive control (T_2). The application of Daconil fungicide (T_2) exhibited minimal suppression of the growth of *R. stolonifer* at 24 HAI and was completely ineffective at 48 and 72 HAI.

The effectiveness of the different concentrations of *B. pilosa* extract against the fungal species isolated from peanut seeds also conforms with the findings of Deba *et al.* (2007 and 2008) and Ashafa and Afolayan (2009).

Conclusion

Based on the study, the aqueous extract from *B. pilosa* inhibits the growth of *A. flavus, A. niger, Fusarium* sp., and *Penicillium* sp. which are commonly associated with corn seeds, but its effectiveness decreases with the time of incubation under *in vitro* conditions. The best antifungal activity is demonstrated in *A. niger*

isolate from 24 to 72 hours after incubation (HAI). Likewise, the fungal isolates from peanut namely, *A. flavus, A. niger, Penicillium* sp., and *Rhizopus stolonifer*, are also sensitive to the aqueous extract of *B. pilosa* with *Penicillium* sp. as the most sensitive fungal species under *in vitro* conditions. However, the antifungal activity of *B. pilosa* extract needs to be evaluated under *in vivo* conditions to further validate the consistency of its performance.

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References

Ashafa OT, Afolaya AJ. 2009. Screening the root extracts from *Biden pilosa* L. var. *radiata* (Asteraceae) for antimicrobial potentials. Journal of Medicinal Plant Research **3(8),** 568-572.

Bartolome AP, Villaseñor IM, Yang W. 2013**.** Botanical properties, traditional uses, phytochemistry, and pharmacology. Evidence-based Complementary and Alternative Medicine **2013,** 1-51.

Deba F, Xuan TD, Yasuda M, Tawata S. 2008. Chemical composition and antioxidant, antibacterial, and antifungal activities of the essential oils from *Bidens pilosa* Linn. var. r*adiata*. Food Control **19(4),** 346-352.

Deba F, Xuan TD, Yasuda M, Tawata S. 2007. Herbicidal and fungicidal activities and identification of potential phytotoxins from *Bidens pilosa* L. var. radiata Scherff. Weed Biology and Management **7(2),** 77-83.

Iqbal CM, Meiyalaghan S, Wijesekara KB, Abeyratne KP. 2001. Antifungal activity from water extracts of some common weeds. Pakistan Journal of Biological Sciences **4,** 843-845.

Sharma B, Srivatva KK, Verma N, Niwas R, Singh M. 2014. Antifungal potential of leaf extract of *Datura stramonium* L. against some important plant pathogenic fungi. Acta Biologica Indica **3(2),** 659-662.

Silva JJ, Cerdeira CD, Chavasco JM, Cintra ABP, Silva CBP, Mendonca AN, Ishikawa T, Boriollo MFG, Chavasco JK. 2014. *In vitro* screening antibacterial activity of *Bidens pilosa* Linn. and *Annona crassiflora* Mart. against oxacillin-resistant *Staphylococcus aureus* (ORSA) from the aerial environment at the dental clinic. Rev. Inst. Med. Trop. Sao Paulo **56(4),** 333-40.

Xuan TD, Khanh TD. 2016. Chemistry and pharmacology of *Bidens pilosa*: an overview. Journal of Pharmaceutical Investigation **46,** 91-132.