
Philippine Ethnobotanicals Show Antifungal Activity against *Candida albicans*, the Causative Agent of Candidiasis

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Ethnobotanicals was used by the Igorot community of Imugan, Nueva Vizcaya, Philippines which were tested for its antifungal activity against *Candida albicans*, a persistent fungus infecting humans. Candidiasis is a major health issue and one of the most frequently hospital-acquired infection globally. Three concentrations of 1250, 750 and 250 ug/ml of ethanol extracts of *Ayapana triplinervis* (Vahl.) R. M. King & H. Rob., *Ageratina adenophora* (Spreng.) R. M. King & H. Rob., *Pittosporum pentandrum* (Blanco) Merr., *Bidens pilosa* L., *Sacandra glabra* (Thunb.) Nakai, *Cestrum nocturnum* L., Lipang daga (local name), *Alstonia scholaris* (L.) R. Br., *Oreocnide trinerevis* (Wedd.) Miq. and *Derris Elliptica* Benth were subjected to disc diffusion assay using corn meal agar against *C. albicans*. Results showed that the ethanol extracts of *Ageratina adenophora*, *Bidens pilosa* and Lipang daga (local name) showed antifungal activity with Lipang daga having the highest fungicidal activity against *C. albicans*. The prevalent antifungal activity of these plants can be tapped for possible elucidation of antifungal agents that may be used for the development of a natural fungicide.

Keywords: Ethnobotanicals, *Candida albicans*, Candidiasis

Introduction

The Philippine archipelago with approximately 7100 islands within extensive coastal waters of 300,000 sq. km (Prigge *et al.*, 2005) is one of the most vitally biologically diverse countries on earth, hosting almost 8900 vascular plant species and enormous number of endemic species. This represents immense number of novel bioactive compounds to discover that can be used to treat diseases.

However, given the tremendous number of plant species in the Philippines, only a small number have been evaluated for their pharmacological potential. One group of the unexplored and untapped plants is the ethnobotanicals, native plant species that grows in the wild and are usually found on areas where the ethnic communities live. The uses of ethnobotanicals are not

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well-documented; moreover, researches on the pharmacological potential of the ethnobotanicals are limited. To attain the maximum potential of these plants as medicine, validation of their pharmacological activities is crucial.

The Philippines is an archeologically rich country hosting diverse ethnic communities with traditional medicinal knowledge. One of the ethnic communities with their distinct culture is the Igorot community that inhabits the Caraballo mountain range on an altitude of more than 3,000 feet in which their domain includes the Kalahan Forest Reserve in Imugan, Nueva Vizcaya, Philippines. This forest serves as a vast resource of plants that are traditionally used as medicine by Igorots. This epitomizes a huge potential to the discovery of drugs that can control the growth pathogenic fungi such as *Candida*.

C. albicans is a persistent fungus infecting humans and similar to bacteria that alters the permeability of the cells. It constitutes a normal flora in humans commonly found on skin, gastrointestinal tract and female genital tract. When the mucosal barriers are disrupted or the immune system is compromised, *C. albicans* can invade and cause virulence (Kourkoumpetis, 2011) causing candidiasis. Candidiasis is a major health issue and is one of the most frequently hospital-acquired infections globally. The most common cases are the oropharyngeal candidiasis which is an over growth of *C. albicans* in the oral cavity, and the vulvovaginitis or vaginal thrush, the yeast infection in female genitals. Globally, about 20% of those receiving chemotherapy for cancer and 20% of those with AIDS also develops Candidiasis. A number of plants were already tested and developed for fungal control. This study reports the fungicidal activity of the ethnobotanicals and the promise of discovering controlling agents against *C. albicans*.

Materials and methods

Collection of Plant Samples

Collection of Plant Samples Leaves of *A. triplinervis*, *A. adenophora*, *P. pentandrum*, *B. pilosa*, *S. glabra*, *C. nocturnum*, Lipang daga, *A. scholaris*, *O. trinerevis* and *D. Elliptica* were collected by hand picking along the trail of Mt. Imanduyan Brgy. Imugan, Sta Fe, Nueva Vizcaya, Philippines with the permission of the Council of Elders. Pressed plants served as voucher specimens and were used for the authentication.

Ethanol Extraction

Plant leaves were cleaned and disinfected to remove foreign bodies and cut into small pieces (2-3 cm) and air dried to make it suitable for grinding then pulverized. Fifty grams of pulverized leaves were soaked in 500 ml 95% ethanol in

a stoppered flask for 72 hours and filtered subsequently. Rotary evaporator was used to remove ethanol from the extract. Extracts obtained were stored in a tightly stoppered sterile amber bottle (Srisawat *et al.*, 2007) and kept refrigerated until use.

Disc diffusion Assay

Three concentrations of 1250, 750 and 250 ug/ml of ethanol extracts of *A. triplinervis*, *A. adenophora*, *P. pentandrum*, *B. pilosa*, *S. glabra*, *C. nocturnum*, Lipang daga, *A. scholaris*, *O. trinerevis* and *D. elliptica* individually were used.

Sterile paper discs (5 mm) were soaked and air dried on sterile petri plates with individually dispensed leaf extracts under a biosafety laminar flow. Prepared media on petri plates of corn meal agar, a selective medium for the cultivation of *C. albicans*, swabbed with fungal culture were used.

Air-dried disc with three concentrations of each plant were individually seeded on plates in a three -concentration per plant manner. Ketoconazole served as positive control while sterile distilled water served as negative control. Plates were incubated at 37 °C for 3-5 days.

Results and Discussion

The ethanol extracts of *Ageratina adenophora*, *Bidens pilosa* and Lipang daga (local name) showed antifungal activity with Lipang daga having the highest fungicidal activity against *C. Albicans* at 1250 ug/ml concentration.

Table 1. Zones of inhibition on *C. albicans* as affected by the ethnobotanical extracts in mm.

	1250 ug/ml	750 ug/ml	250 ug/ml	+ Control	- Control
<i>A. triplinervis</i>	0 ^a	0 ^a	0 ^a	9.5 ^b	0 ^c
<i>A. adenophora</i>	10.1^a	7.7^b	6.8^c	10.4^d	0^e
<i>P. pentandrum</i>	0 ^a	0 ^a	0 ^a	11.3 ^b	0 ^c
<i>B. pilosa</i>	8.0^a	7.5^b	6.1^c	11.4^d	0^e
<i>S. glabra</i>	0 ^a	0 ^a	0 ^a	8.2 ^b	0 ^c
<i>C. nocturnum</i>	0 ^a	0 ^a	0 ^a	9.0 ^b	0 ^c
Lipang daga	12.3^a	10.4^b	7.8^c	13.8^d	0^e
<i>A. scholaris</i>	0 ^a	0 ^a	0 ^a	12.8 ^b	0 ^c
<i>O. trinerevis</i>	0 ^a	0 ^a	0 ^a	11.2 ^b	0 ^c
<i>D. Elliptica</i>	0 ^a	0 ^a	0 ^a	11.9 ^b	0 ^c

*Superscripts of different letters indicate significant difference among the treatments.

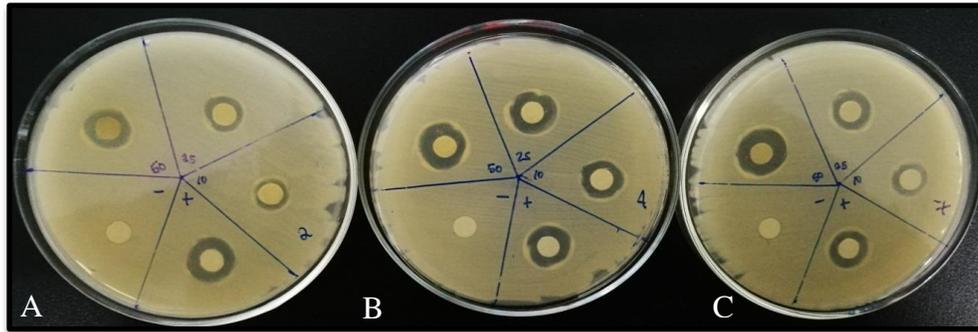


Figure 1. Disc diffusion assay of A) *A. adenophora* , B) *B. pilosa* and C) Lipang daga (Common name)

The efficacy of plant extracts may be due to the presence of several primary and/or secondary metabolites such as tannins, saponins, lectins, flavonoids, alkaloids, tannins, polyenes, terpenoids, glycoside and other aromatic compounds which are known antifungal agents. Plant-derived metabolites are antimicrobial in nature. These phytochemicals also induce immunity that indirectly reduces the risk of infections such as those in candidiasis and other *Candida*-caused diseases. These phytochemical components are responsible for breaking down build-up lining on the intestinal walls that enables the eradication of *Candida albicans*. In this sense, they function as natural antibodies and effective when used to treat fungal infection.

Ageratina adenophora principally contains alkaloids, glycosides, saponins and coumarins (Bandawane *et al.*, 2011). *Bidens pilosa* contains active compounds such as flavonoids and polyenes, terpenoids, phenylpropanoids and other aromatic compounds (Stuart, 2013). These phytochemical compounds have known antifungal activity that inhibits the growth and virulence of fungi, including *C. albicans*. Among the plants tested, Lipang Daga (local name) is the most effective plant extract against *C. albicans*. However, this plant has not been fully studied in terms of phytochemical analysis.

Several biological activities were also discovered on this group of ethnobotanicals. These ethnobotanicals have antibacterial properties against *E. coli* (Gabriel *et al.*, 2015). Other biological activities include anti proliferative and cytotoxic activities against human cancer cell lines such as colon cancer, breast cancer and lung cancer, quorum sensing inhibition properties (Benito *et al.*, 2015; Barrogo *et al.*, 2015; Padilla *et al.*, 2015), analgesic (Gabriel *et al.*), anti-inflammatory (Gabriel *et al.*, 2015), anti-gout (Jose *et al.*, 2015), anti-diabetic (Alfonso *et al.*, 2015) and antioxidant (Divina *et al.*, 2015).

This study on the antifungal activity of Philippine ethnobotanicals against *Candida albicans* add up to the discovered biological activities of these plants. The prevalent antifungal activity of *Ageratina adenophora*, *Bidens pilosa* and Lipang daga (local name) may be tapped for possible elucidation of antifungal agents that may be considered for the development of drugs for the control of fungal pathogens such as *C. albicans*.

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