

***In Vitro* Antifungal Activity and Phytochemical Screening of *Gouania javanica* Miq. Leaves**

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Abstract

Gouania javanica Miq. leaves were collected from the users in Nagbukel, Ilocos Sur. The plant was authenticated at the National Museum Botany Division, Manila (November 15, 2007, Control No. 578). The weighed 200 grams of fresh plant material were extracted with ethanol and concentrated to 20 ml.

Antifungal Assay of the extract. The following microorganisms were employed in the assay: *Candida albicans*, *Trichophyton mentagrophytes* and *Aspergillus niger*. These were obtained from the culture collection of the Natural Science Research Institute (NSRI), University of the Philippines, Diliman, Quezon City.

Screening for antifungal activity. The Kirby-Bauer Disk Diffusion Method was used for this specific part.

The Sabouraud agar plates were seeded with the inoculum using a sterile cotton swab. The entire surface of the agar was streaked evenly in all directions. The plates were incubated at room temperature for 72 hours, after which the diameter of the zones of inhibition were measured using a Vernier caliper. The antifungal testing was done in three replications.

Phytochemical analysis. The extracts were subjected to phytochemical screening to detect the presence of some secondary plant metabolites following standard procedures from the Chemistry and Pharmacological Division, Department of Science and Technology, Bicutan, Taguig City, Metro Manila.

The leaf extract showed strong (+++) antifungal activity against *Aspergillus niger* with a mean diameter of zone of inhibition of 21.16 mm; moderate (++) antifungal activity against *Candida albicans* with a mean diameter

of zone of inhibition of 12.08; and weak (+) antifungal activity against Tricophyton mentagrophytes with a mean diameter of zone of inhibition of 8.55.

Phytochemical analysis of the Gouania leaf extract revealed the presence of the following secondary metabolites: flavonoids, saponins, triterpenes, glycosides, and alkaloids. The presence of alkaloids which turned out to be moderate (++) is just a manifestation that this plant species can be a very good source of medicine. Since some of the 30 known alkaloids are used in medicine today, the researchers find it important that the specific type of alkaloid present in the leaves of the Gouania plant should be isolated in the near future. Further, flavonoids present in the leaves is an indication of the plant's antiviral, antifungal, anti-inflammatory and cytotoxic activities (Copal, 1992). Saponins, triterpenes and glycosides are also very important compounds, thereby making this plant species a very good source of medicine and other important products.

Introduction

Background of the Study

A knowledge of the chemical constituents of plants is desirable not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of such economic materials as tannins, oils and gums, precursors for the synthesis of complex chemical substances. In addition, the knowledge of chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies.

The use of medicinal plants predates the introduction of antibiotics and other modern drugs in the Philippines and in almost all countries of the world. Herbal medicine has been widely used and formed an integral part of primary health care in almost all parts of the Philippine islands. Traditional medical practitioners in the country use a variety of herbal preparations to treat different kinds of microbial diseases.

According to Akinyemi et al (2007), herbal medicine has been shown to have genuine utility and about 80% of rural population depends on it as primary health care. Over the years, the World Health Organization (WHO) advocated that countries should interact with traditional medicine with a view to identifying and exploiting aspects that provide safe and effective remedies for ailments of both microbial and non-microbial origin.

Gouania javanica Miq. belongs to the Family Rhamnaceae. The stem is used in traditional folk.lore medicine to cure mouth thrush, a fungal infection. The plant is found

from Northern Luzon to Mindanao from thickets at low and medium altitudes. It is also reported in India to the Mascarene Islands, Indochina and Malaya. The plant is an unarmed, climbing shrub. The leaves are alternate, somewhat hairy, ovate, six to 10 cm long and 4-5 cm wide, with pointed tip and slightly heart-shaped base. The flowers are small, greenish or whitish and borne in axillaries or terminal panicles. The fruit is smooth, with narrow wings. The plant is known from different dialects such as Litiran (Tag); Lina-a (Bag); Rungo-rungo (Ilk) and pahampak (Pam) (Quisumbing, 1978).

In Nagbukel, Ilocos Sur, people in the locality cut a portion of the plant's stem and blow it at one end. The bubbles coming out from the other side is directly applied to mouth thrush, a kind of candida infection.

A study conducted by delos Santos and Cellona (2007) on the antimicrobial activity of *Gouania javanica* Miq. stem extract found out that the ethanol extract exhibited a strong inhibition on the fungus *Candida albicans* as compared to a weak effect against the bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosa*, in terms of the average diameters of growth inhibition.

This study served as a basis for the researchers to further conduct a study on the antifungal activity of other parts of the plant, the leaves specifically, to find out if it could be comparable to that of the stem, or even a better antifungal agent.

Objectives

This study was conducted to determine the presence of chemical constituents, specifically the secondary metabolites from the ethanol extract of *Gouania javanica* Miq. leaves. Further, the study also evaluated the antifungal activity of the ethanol extract of *Gouania javanica* leaf against *Candida albicans*, *Aspergillus niger* and *Tricophyton mentagrophytes*.

Scope and Limitation

The study was limited to the determination of the presence of secondary metabolites in the ethanol extract of *Gouania javanica* leaves, as well as to the evaluation of the antifungal activity of the leaf extract against *Candida albicans*, *Aspergillus niger* and *Tricophyton mentagrophytes*. Comparison of the antifungal activity of the leaf extract to that of a standard antibiotic was not conducted due to the unavailability of the specific antibiotic to be used.

The experiment was conducted at the Natural Products Chemistry and Biotechnology Laboratory, Science Complex I, University of Northern Philippines, Vigan City, Ilocos Sur from January – March 2008.

Review of Related Literature and Studies

According to the World Health Organization (WHO) more than 80% of the world's population relies on traditional medicine for their primary health care needs. Use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. A vast knowledge of how to use the plants against different illnesses may be expected to have accumulated areas where the use of plants is still of great importance. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds (pubmedcentral.nih.gov/articlerender.fcgi?tool=pubmed&pubmedid=17042964-61k)

The Republic Act 8423 established the Philippine Institute of Traditional and Alternative Health Care (PITHAC). This law being referred to as Alternative Medicine Law is expected to promote and develop the country's traditional or folk knowledge of healing and medicinal practices especially the use of medicinal plants. One of the objectives of this act is to encourage scientific research and develop traditional and alternative health care systems that have direct impact on public health care (Bernardo, 1999).

Rungo-rungo is scientifically known as *Gouania javanica*. It belongs to Family Rhamnaceae. The plant is found from Northern Luzon to Mindanao in thickets at low medium altitudes. It is an unarmed, climbing shrub. The leaves are alternate, somewhat hairy, ovate, 6 to 10 centimeters long and 4 to 5 centimeters wide, with pointed tip and slightly heart-shaped base. The flowers are small, greenish or whitish and borne on axillary or terminal panicles. The fruit is smooth, with narrow wings (Quisumbing, 1978).

Delos Santos and Cellona (2007) found out in their study on the antimicrobial activity of *Gouania javanica* Miq. that the stem extract strongly inhibited the growth of the fungus *Candida albicans* in terms of the average diameters of growth inhibition.

Florendo and Segismundo (2006) conducted a study on the antifungal property of Kuchai bulb extract on the three test organisms namely: *Candida albicans*, *Aspergillus niger* and *Tricophyton mentagrophytes*. Using ethyl alcohol as solvent by measuring their

diameters of growth inhibition, the bulb extract was strong(+++) on both *Candida albicans* and *Aspergillus niger* and moderate(++) on *Tricophyton mentagrophytes*.

The antifungal property of kuchai (*Allium tuberosum* Rotler ex. Spreng) bulb extract was tested on *Candida albicans*, *Aspergillus niger* and *Tricophyton mentagrophytes* by measuring their diameters of growth inhibition. Using ethyl alcohol and water as solvents, the leaf extract was strong(+++) on the growth of *C. albicans* and *A. niger*; moderate(±) on *Tricophyton mentagrophytes* using ethyl alcohol as solvents and weak(+) on *C. albicans* and *A. niger* using water as solvent. The leaf extract had a negative (-) effect on *T. mentagrophytes* (Bonifacio et al, 2006).

Ailments and/or diseases are often caused by certain species of microorganisms that include bacteria, fungi, and protozoa. Three species of pathogens namely *Candida albicans*, *Aspergillus niger* and *Tricophyton mentagrophytes* are among the pathogenic microorganisms tested for their reaction, in terms of growth inhibition with plant extract.

Fungal infections are diseases caused by the growth of fungi in or on the body. In most healthy people fungal infections are mild, involving only the skin, hair, nails, or other superficial sites, and they clear up spontaneously. They include the familiar ringworm and athlete's foot. Fungi can also invade internal organs of the body, especially the lungs, where the infections resemble pneumonia or pulmonary tuberculosis. These infections usually occur in people whose immune system has been suppressed by diseases such as acquired immunodeficiency syndrome (AIDS), by anticancer drugs, or by radiation; patients being treated with steroid hormones and people with diabetes; and those being treated with antibiotic drugs for a long time. Two fungi often found in such cases are *Cryptococcus* and *Aspergillus*, which are called opportunistic pathogens. Many drugs are available for treating fungal infections. These include both intravenous and oral drugs, and many agents are available for topical (local) application (Microsoft Encarta Premium, 2007).

Candida albicans is a diploid asexual fungus that live in the human mouth and gastrointestinal tract. Under normal circumstances, *C. albicans* lives in 80% of the human population with no harmful effects, although overgrowth results in candidiasis. Candidiasis also known as "thrush", is a common condition that is usually easily cured in people who are not immuno-compromised. To infect host tissue, the usual unicellular yeast-like form of *Candida albicans* reacts to environmental cues and switches into an invasive, multicellular filamentous form (en.wikipedia.org/wiki/candida_albicans).

Aspergillus niger is a fungus and is one of the most common species of the genus *Aspergillus*. It causes a disease called black mold on certain fruits and vegetables such as grapes, onions, and peanuts, and is a common contaminant of food. Some strains of *A. niger* have been reported to produce potent mycotoxins called ochratoxins. The fungus is less likely to cause human disease than some other *Aspergillus* species, but if large amounts

of spores are inhaled, a serious lung disease, aspergillosis can occur. (http://en.wikipedia.org/wiki/Aspergillus_niger).

Trichophyton mentagrophytes is a keratinophilic fungus belonging to a homogeneous group of fungi called the dermatophytes. The dermatophytes cause a variety of cutaneous infections in humans and animals. The organism has been recovered from a variety of sources such as soil, floor of swimming pools, hairs of wild boar, cats and dogs, farm animals, foot **wears**, shower stalls and from human toe webs without clinical lesions. *T. mentagrophytes* breaks down keratinous substrates by both chemical and mechanical ways. Five different keratinolytic enzymes from ten strains of *T. mentagrophytes* have been isolated. These enzymes are known to play a role in pathogenesis of infections caused by these organisms in both humans and animals (en.wikipedia.org/wiki/trichophyton_mentagrophytes).

Methodology

Gouania javanica Miq leaves were collected from the users in Nagbukel, Ilocos Sur. The plant was authenticated at the National Museum Botany Division, Manila (November 15, 2007, Control No. 578). The weighed 200 grams of fresh plant material was extracted with ethanol and concentrated to 20 ml for the antifungal screening.

Screening for antifungal activity. The Kirby-Bauer Disk Diffusion Method was used for this specific part.

The following microorganisms were employed in the assay: *Candida albicans*, *Aspergillus niger* and *Trichophyton mentagrophytes*. These were obtained from the culture collection of the Natural Science Research Institute (NSRI), University of the Philippines, Diliman, Quezon City.

The procedures used were adopted from the Manual on Extraction Procedures and Microbial Assay of Medicinal Plants by Capal et al. (1998) and the Guide Book to Plant Screening: Phytochemical and Biological by Guevarra (2005).

The Saboraud agar plates were seeded with the inoculum using a sterile cotton swab. The entire surface of the agar was streaked evenly in all directions. The plates were incubated at 27°C for 24 hours for the yeast and at 27°C for 72 hours for the molds, after which the diameter of the zones of inhibition were measured using a Vernier caliper. The following interpretative range of standard zone was adopted from Ontengco (1992).

Zone of Inhibition	Inhibitory Activity
>17	+++, strong
12–16	++, moderate
7--11	+, weak
6 or 0	-, negative

The antifungal activity testing was done in three replications.

Phytochemical analysis. The *Gouania* leaf extract was subjected to phytochemical screening to determine the presence of secondary metabolites following standard procedures from the Chemistry and Pharmacological Division, Department of Science and Technology, Bicutan, Taguig City, Metro Manila.

Results and Discussion

Table 1. Antifungal Activity of *Gouania Javanica* Leaf Extract Against the Three Test Organisms.

Test Organism	Replication	Trial	Diameter of Inhibition (Mm)	Mean	Grand Mean	Anti-Fungal Activity
<i>Candida alb/cans</i>	1	1	12.0	11.85	12.08	++
		2	11.0			
		3	12.55			
		C	6.0			
	2	1	12.25	12.58		
		2	12.75			
		3	12.75			
		C	6.0			
	3	1	11.75	11.82		
		2	10.85			
		3	12.85			
		C	6.0			
<i>Aspergillus niger</i>	1	1	21.75	20.58	21.16	+++
		2	21.0			
		3	19.0			
		C	6.0			
	2	1	21.75	21.08		
		2	20.0			
		3	21.50			
		C	6.0			
	3	1	24.5	21.83		
		2	17.0			
		3	24.0			
		C	6.0			

Table 1 continued

Test Organism	Replication	Trial	Diameter of Inhibition (mm)	Mean	Grand Mean	Anti-Fungal Activity
<i>Tricophyton mentagrophytes</i>	1	1	8.85	8.58		
		2	8.35			
		3	8.55			
		C	6.0			
	2	1	9.35	8.60	8.55	+
		2	8.45			
		3	8.0			
		C	6.0			
	3	1	8.15	8.46		
		2	9.0			
		3	8.25			
		C	6.0			

Legend:

C- Control (++)-- strong (++) - Moderate (+) - Weak

Antifungal activity testing. Table 1 shows the antifungal activity of *Gouania javanica* leaf extract against the test organisms.

The leaf extract showed strong(+++) antifungal activity against *Aspergillus niger* with a mean diameter of zone of inhibition of 21.16 mm; moderate(++) antifungal activity against *Candida albicans* with a mean diameter of zone of inhibition of 12.08; and weak (+) antifungal activity against *Tricophyton mentagrophytes* with a mean diameter of zone of inhibition of 8.55.

Phytochemical analysis. The result of phytochemical analysis is presented in Table 2.

Table 2. Result of the Qualitative Test of *Gouania javanica* Miq.

Component	Qualitative Test	Result
Alkaloids	Mayer's test	Moderate (++)
Flavonoids	Color test	Traces(+)
Glycosides	Fehling's test	Traces(+)
Saponins	Froth test	Traces(+)
Sterols	Liebermann Burchard test	Negative (-)
Tannins	Gelatin test	Negative (-)
<i>Triterpenes</i>	Liebermann Burchard test	Traces(+)

Phytochemical analysis of the *Gouania* leaf extract revealed the presence of the following secondary metabolites: flavonoids, saponins, triterpenes, glycosides, and alkaloids. The presence of alkaloids which turned out to be moderate (++) is just a manifestation that this plant species can be a very good source of medicine. Since some of the 30 known alkaloids are used in medicine today, the researchers find it important that the specific type of alkaloid present in the leaves of the *Gouania* plant should be isolated in the near future. Further, flavonoids present in the leaves is an indication of the plant's antiviral, antifungal, anti-inflammatory and cytotoxic activities (Capal et. al., 1992). Saponins, triterpenes and glycosides are also very important compounds, thereby making this plant species a very good source of medicines and other important products.

Conclusion

This study has revealed the presence of secondary metabolites like flavonoids, saponins, triterpenes, glycosides, and alkaloids in the leaves of *Gouania javanica*. It has further confirmed that the leaf extract could be used for the treatment of infections caused by the fungi *Aspergillus niger* and *Candida albicans*. The result on *Candida albicans* lend credence to the folkloric use of this plant in treating microbial infections and shows that *Gouania javanica* could be exploited for new potential antibiotics.

Recommendations

1. The Minimum Inhibitory Concentration (MIC) should be determined.
2. The same study should be conducted making use of a standard antibiotic as the positive control.

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