Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 1002 - 1014
Received 05 March 2021; Accepted 01 April 2021.

Efficacy of Chromolaenaodorata (Wellawel) Extract in Inhibiting the Growth of Selected Fungi

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ABSTRACT

The realization that synthetic medicine, like antibiotics, has adverse effects made man recognize the importance of medicinal plants. Plants possess a wide array of secondary metabolites, such as tannins, glycosides, alkaloids, and flavonoids, that have antimicrobial properties in vitro.

Chromolaenaodorata or Wellawel is believed in folk medicine to have medicinal properties. The different parts of the plant, like the young leaves, mature leaves, and the flowers, were studied. The study revealed that of the three parts, the former two have the potential of becoming an antifungal agent against four species of fungi.

In the qualitative determination of the phytochemical contents, flavonoid was found in all the plant extracts, while tannins and alkaloids were both demonstrated in the leaf extracts. Of the six fungi, Candida albicans, Aspergillusniger, and Penicilliumnotatum were found most sensitive to the young leaf extract, mature leaf extract, and flower extract, respectively, with a minimum inhibitory concentration (MIC) of 12.5mg/ml each. The MIC means that even at a low concentration of the extract, it is enough to cause inhibitory activity. However, the activity (3 mm each) is said to be resistant in terms of its overall effect.

The researchers utilized the Kirby Bauer Disc Diffusion method to determine the sensitivity of the six fungal strains against the different concentrations of the three extracts, and to the control groups. The study revealed that only the young and mature leaf extracts at 100% concentration have the potential of an antifungal agent against C. albicans, Aspergillusniger, Microsporumcanis, and Penicilliumnotatum. Both extracts have no antifungal activity against the other two fungi – T. mentagrophytes and C. guilliermondii. Likewise, the flower extract had no antifungal activity against all the fungi tested.

The researchers concluded that the ethanolic extracts of the Wellawel leaves are potential antifungal agents against the susceptible fungal strains. Finally, the researchers forward the following recommendations: (1) to test the extract with other fungal species; (2) to employ another solvent for extraction that could effectively extract all of the active substances present in the different parts of the plant; (3) to formulate a pharmaceutical product like an ointment or cream made from the Wellawel leaf extract as an antifungal agent; and (4) to encourage people to cultivate Wellawel in their backyard.

Keywords:
Efficacy, Chromolaenaodorata, antifungal, plant extract

Introduction

Plants have traditionally served as man's important weapon against disease-producing organisms. Medicinal plants and their active constituents can alleviate human diseases. No one would seriously challenge the fact that man is still largely dependent on plants in treating his ailments. Approximately 80% of the people in developing countries rely chiefly on traditional medicine for their primary health care needs, of which the majority involve the use of plant extracts or their active ingredients (WHO, 2004).

With the development of modern medicine, synthetic drugs, and antibiotics, the importance of plants as raw materials for drugs decreased considerably in the early seventies. Now, the pendulum is swinging back, and the global significance of medicinal plants is currently recognized. The whole world has been swept by a "Green Wave" during the last three decades. With the realization of health hazards and toxicity associated with the indiscriminate use of synthetic drugs and antibiotics, there has been a general acceptance that anything in nature is safer than synthetics. It is also noteworthy that some of the essential drugs, which have

revolutionized modern medical practices, have almost all been isolated from plants. It is of prime importance to conduct researches on higher medicinal plants, which are known to be potential sources of some natural products such as the phytochemicals.

Plants are rich in a wide variety of secondary metabolites, such as tannins, glycosides, alkaloids, and flavonoids, which have been found in vitro to have antimicrobial properties (Cowan, 1999). Since many of these compounds are currently available as unregulated botanical preparations and their use by the public is increasing rapidly, clinicians need to consider the consequences of patient's self-medication with these preparations.

Medical scientists had tried to look for alternative and novel ways to treat diseases. These innovations are even more efficient and effective as they are all subjected to clinical trials. These are all meant to impact the medical world.

The study of microorganisms is a fascinating subject. It impacts one's daily life in various ways. Organisms live on land and bodies of water. They are necessary for many industries. They are essential in the cycling and recycling of elements such as carbon, oxygen, and nitrogen. They provide most of the oxygen in our atmosphere. They are used to clean up toxic wastes and also in genetic engineering, as well as in gene therapy. However, many of these microorganisms also cause diseases to humans.

In recent years, news and other reports about flesh-eating bacteria, mad cow disease, superbugs, black molds in buildings, West Nile virus, anthrax, smallpox, and others flood the newsfeed. There were even meat recalls due to *Escherichia coli* contamination and epidemics of meningitis, hepatitis, influenza, and diarrheal diseases (Burton et al., 2011).

The promise of antibiotics seems to be fading. The rising tide of antibiotic-resistant bacteria that cause serious diseases has made them untouchable by modern medicine and resistant to every single antibiotic in today's era against infection. Patients infected with these resistant bacteria are dying like it was before. Some fear that the world may be returning to an era without the benefit of modern antibiotics.

The discovery of alternative medications is relevant since modern science acknowledges the adverse effects of synthetic medicines. At one point in time, people believed that drugs originated from synthetic sources. However, despite the phenomenal development of new drugs from synthetic sources and the discovery of antibiotics as key therapeutic agents, plants continue to provide raw materials for some of the essential drugs. And one of the plants believed to cure skin illnesses is Wellawel, named *Chromolaenaodorata Linn*. It is a coarse, erect, perennial, branched shrub found locally abundant throughout the Philippines. It can grow to as high as 2.5 meters with woody branches. Leaves are simple, opposite, without spicules, but with serrated edges. When crushed, it produces a characteristic odor, hence the word odorata.

Community folks believed that the plant has healing properties. It is a common practice to crush its young leaves and apply on skins to relieve itchiness and pruritus. However, there is no scientific basis for all these claims.

There are no published studies on the antifungal effect of Wellawel known to the researchers. Thus, the researchers conducted this study to determine the antifungal activity and efficacy of the *Chromolaenaodorata* against the six fungi.

This study contributed to the discovery of new species of plants with medicinal properties. Likewise, it scientifically confirmed its use among folks in the communities. It is an initial step towards the development of an antifungal drug from *C. odorata.*

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 1002 - 1014
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Study Objectives

This study determined the efficacy of *C. odorata* in inhibiting the growth of selected fungi. Specifically, it determined the phytochemical constituents present in the different parts of the Wellawel plant; the inhibitory activity as measured by the mean zones of inhibition (ZOI) of the following concentrations of the Wellawel extracts (young leaf, mature leaf, and flower extracts) on *Candida albicans, Candida guillermondii, Aspergillusniger, Trichophytonmentagrophytes, Microsporumcanis,* and *Pennicilliumnotatum*: 25%, 50%, 75%, and 100%; the minimum inhibitory concentration of the Wellawel extracts; if a significant difference exist in the zones of inhibition of the different fungi subjected to different treatments; and if a significant difference exist in the zones of inhibition by each concentration of the three extracts from each other.

Materials and Methods

Research Design

This study utilized the experimental research design. The extraction of the different parts of the plant, as well as the fungal sensitivity assay were conducted in a Biosafety Level 2 laboratory in Manila, Philippines.

Preparation of Plant Materials

The researchers collected the plant samples in the province of Abra, Northern Philippines. It includes the young leaves, the mature leaves, and the flowers of the *Chromolaenaodorata*. The Bureau of Plant Industry of the Department of Agriculture, verified and authenticated the plant samples. The rest of the plant samples were washed clean with tap water and finally with distilled water. The leaves (young and mature) and the flowers were shade-dried separately for one week and manually pulverized.

Preparation of the Plant Extracts

The researchers suspended separately the pulverized 250 grams young leaves, 250 grams mature leaves, and 250 grams flowers in Erlenmeyer flasks containing 500mL of 95% ethyl alcohol. The preparations were mixed well, allowed to stand for 48 hours, filtered, and subjected to the Rotary evaporator machine to evaporate the alcohol until a syrupy solution is left. The resulting solution is considered 100% extract.

Phytochemical Analysis (Kaur et al., 2011)

The researchers tested the three extracts for the presence of active constituents:

a.Test for Alkaloids – Meyers test

The researchers placed five hundred microliters (500 µL) each of the three extracts in separate test tubes. Two (2) mL of 2M HCl was added and heated with stirring for about 5 minutes. After cooling, the researchers added 0.25 g of NaCl, stirred and filtered to a volume of 2.5 ml, after which, 1-2 drops of Meyer's reagent was added. A white precipitate indicated a positive result.
In the test, all extracts were tested positive for Alkaloids.

b. Test for Flavonoids

Bate-Smith and Melcalf Tests for Leucoanthocyanins

A portion of the crude extract equivalent to 2 grams of each of the plant parts was evaporated to dryness over a water bath and cooled to room temperature. The researchers treated the residue with 2 mL of hexane, mixed and decanted. The researchers removed the hexane and dissolved the residue in 4 mL of 80% ethyl alcohol. The resulting solution was divided into three portions,

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labelled A, B, and C. The researchers added 0.5 ml concentrated HCl to tube A, and warmed for 15 minutes in a water bath. A red or violet color indicated the presence of leukoanthocyanin. The test revealed that the young and the mature leaf extracts have a higher concentration of leukoanthocyanins compared to the flower extract as manifested by a more intensed end color.

Wilstatter "cyaniding" Test

The researchers added a pinch of magnesium turnings to 0.5 mL of concentrated HCl on test tube B. They compared the color change of test tube B with the blank (test tube C). Then, an equal volume of water and 1 mL of acetyl alcohol were added simultaneously to both test tubes. The mixture was shaken and allowed to stand. The formation of a red color indicated the presence of cyanidin.

c. Test for saponins

The researchers dissolved and mixed in a test tube a portion of each of the dried extract in hot water until it becomes frothy. Saponin is indicated by the presence of froth which persisted for at least 30 minutes.

In the experiment conducted, there was no frothy observed indicating the absence of saponin in any of the extracts tested.

d.Test for Tannins

A portion of the dried extract from each of the three samples was dissolved in hot water and filtered. To the filtrate was added two drops of ferric chloride solution. A blue-black color or precipitate indicated the presence of tannins.

The leaf extracts, but not the flower extract, demonstrated the presence of tannins.

Preparation of Culture Media (Difco, 2003)

The researchers utilized Potato Dextrose agar (PDA) in the culture of the fungal species. They prepared it by dissolving 39 grams of the agar in one (1) liter of distilled water. The preparation was sterilized by autoclaving at 15 psi for 15 minutes.

The disc susceptibility testing utilized the Mueller Hinton Agar with Glucose and Methylene blue (MHA-GMB). The researchers prepared by dissolving 28.5 grams powder in 750ml of distilled water. The mixture was subjected to heating on a hot plate with a constant swirling at a regular interval to dissolve the powder. Once dissolved, the solution was subjected to sterilization using an autoclave at 121^0C for 15 minutes and 15 pounds per square inch pressure.

Preparation of Test Plates

The researchers transferred the sterile plates to the alcohol-swabbed surface of a table where 15 mL of the culture media was poured separately into each, allowed to solidify, and placed in a temperature-controlled compartment for use in the succeeding steps of the experiment.

Preparation of the Inoculum

Candida albicans(ATCC 2168), *Candida guilliermondii*(IDTI 4587), *Trichophytonmentagrophytes*(CPH 4193), *Microsporumcanis*(IDTI 140009), *Penicilliumnotatum,* and *Aspergillusniger*(IDTI 5524) are the fungi used in the study. These six (6) fungi were sub-cultured in a Potato Dextrose Agar (PDA) medium and incubated at room temperature for 3-5 days. One loopful of each of the test organism was inoculated aseptically into test tubes containing 5mL Normal Saline Solution (NSS). The researchers compared the turbidity of the suspension using 0.05 McFarland standard.

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Antifungal Susceptibility Testing

The antifungal activity of the Wellawel extracts was determined using the Kirby Bauer Disc Diffusion Method.

Using a sterile cotton swab, the researchers inoculated the fungal inoculums uniformly into the previously prepared MHA-GMB agar plates. Then paper discs containing the different concentrations of the three extracts were distributed at equal distances on the surface of the agar plates. Similarly, positive control and negative control disks were used side by side with the test disks. The control solutions used in the experiment were sterile distilled water (negative control) and 10µg Fluconazole and 10µg Itraconazole (positive control agents).

The different plates were incubated in an inverted position at 25^0C-30^0C for 24 hours for *Candida albicans* and *Candida guilliermondii*, 48 hours for *Aspergillusniger, Microsporumcanis and Penicilliumnotatum*and seven days for *Trichophytonmentagrophytes.*

Interpretation and Recording of Antifungal Activity

After the incubation period, the researchers observed for the presence of clear zones of inhibition around the paper discs for all the samples tested. The presence of a zone of inhibition indicated a positive antifungal activity, while the failure of the disc to exhibit a zone of inhibition indicated the absence of antifungal activity. Each of the zones of inhibition was measured in millimeters (mm) using a Verniercaliper.

Table 1 below presents the interpretative rating used to describe the zones of inhibition:

Table1. Descriptive Rating of the Zones of Inhibition

Zone of Inhibition	Descriptive Rating
=/<9 mm	Resistant
10-13 mm	Weak
14-17 mm	Moderate
=/>18 mm	Strong

Determination of the Minimum Inhibitory Concentration (MIC) of the Wellawel Extract

A serial dilution of different concentrations of the extract was prepared and tested for the different test organisms as follows: 100mg/mL, 50mg/mL, 25mg/mL, 12.5mg/mL, and 6.25mg/ml, and so on. The purpose of this was to determine the lowest concentration at which the extracts would give antifungal activity against the most susceptible fungi. The researchers used the macro-tube dilution test, as recommended by Delost (2004) in the determination of the MIC of the plant extract.

Statistical Analysis

The Mean described the zone of inhibition while the Standard Deviation determined the dispersion of the zones of inhibition from the mean of each experimental and control group. The Analysis of Variance (ANOVA) compared the zones of inhibition exhibited by each concentration of each extract across all the fungi and compared the zones of inhibition manifested by each concentration of the three extracts from each other, including the control.

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Biosafety Clearance

Since this study involved the handling of pathogenic fungi, it posed a considerable risk to the researchers and the environment. The researchers observed the universal precaution strictly as required-for when handling infectious materials. They implemented strict measures in the handling of the fungus during the preparation of inoculums, bioassay, and the disposal of the waste products of the activity.

RESULTS AND DISCUSSIONS

Phytochemical Constituents Present in Wellawel Extract

The researchers performed qualitative phytochemical analysis to detect for the presence of the phytochemical constituents in the different extracts of the plant. As gleaned from Table 2, the extracts from Wellawel's young and mature leaves contain the same phytochemical contents (tannins, alkaloids, and flavonoids) while the flower extract contains alkaloids and flavonoids only.

Table 2. Phytochemical Components of the Various Parts of the Wellawel

Phytochemicals	*Chromolaenaodorata* (Wellawel)		
	Young Leaf Extract	Mature Leaf Extract	Flower Extract
Tannins	(++)	(++)	(-)
Saponins	(-)	(-)	(-)
Alkaloids	(+)	(++)	(+)
Flavonoids	(++)	(++)	(+)

Legend: (-) – Absent (+) – Weak (++) – Moderate (+++) - Strong

In the case of flavonoids, the researchers performed the Bate-Smith and Metcalf test for leucoanthocyanins and the Wilstatter test for cyanidin. Both tests revealed that all extracts contain both classes of flavonoids.

Tanins were found positive in the leaf extracts – both the young and mature. Tannins have an antiseptic effect because of the phenolic part in its molecular structure. Phenols possess antiseptic properties and prevent infections. Tannins can exclude microorganisms from a particular area so that the area will be sterile. The mechanism for this is their ability to precipitate protein, rendering them resistant to proteolytic enzymes. In living tissues, they have "astringent" action. They act as astringent in gastrointestinal tracts and on skin abrasions. In the treatment of burns, the proteins of the exposed tissues precipitate and form a mild antiseptic, protective coat under which the regeneration of new tissues may take place. They act as antidiarrheal and employed as an antidote in the treatment of poisoning by heavy metals, alkaloids, and glycosides.

Both the young and mature leaf extracts contain alkaloids, as manifested by the precipitation that occurred. Alkaloids are known to be antimicrobial, but most of its classes are toxic, and they do not feature strongly in herbal medicine. Still, they have always been important in the allopathic system, and in homeopathy, where the dose rate is low to cause harm. Taesotikul et al. (2003) studied carregeenan-induced rat paw edema, yeast induced hyperthermia in rat and writhing response induced by acetic acid in mice. The study showed that alcoholic extracts from the stems of *Tabermaemontanapandacaqui*Poir have significant anti-inflammatory, antipyretic, antinociceptive activities due to the alkaloidal components.

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All the extracts showed negative results for saponin, as manifested by the absence of froth formation. Saponins are a group of plant glycosides with attached water-soluble sugars to the steroid (C27) or pentacyclictriterpenoid (C30), a glycose. They form foams in water and cause red cells to disintegrate. They are related to cardiac glycosides. Saponins are toxic to insects and parasites, and they may confer protection from diseases caused by them.

Zones of Inhibition of the Different Extracts

Young Wellawel Leaf Extract

Table 3 shows the zone of inhibition induced by the different concentrations of the young Wellawel extract on the test fungi. Generally, as the concentration of the leaf extract increases, the zone of inhibition also increases. The immature Wellawel leaf extract showed a potential of inhibiting the growth of *Candida albicans, Aspergillusniger, Microsporumcanis, and Penicilliumnotatum* at 100% concentrations with a computed mean value of 15.5mm, 10.2mm, 10.7mm, and 13.7mm respectively. Although it was not as effective as the positive control agents (Fluconazole and Itraconazole), it has a promising antifungal activity as manifested by their respective zones of inhibition.

The 25%, 50%, and 75% concentrations of the extracts against the four fungi specified above have antifungal activity but are considered weak. Furthermore, the extract did not show any inhibition pattern on the growth of *C. guilliermondii* and *T. mentagrophytes* at the different concentrations used, as revealed by the computed mean value of 0.00.

Table 3. Mean Zones of Inhibition Induced by Different Concentrations of the Young Leaf Extract on the Six Fungi

		Distilled water	25%	50%	75%	100%	Fluconazole (10µg)
Candida Albicans	Mean (mm)	0.0 NIA	3.0 Weak	6.0 Weak	8.0 Weak	15.5 Moderate	20 Strong
	SD	0.0	1.0	1.4	2.0	2.0	2.2
Candida guilliermondii	Mean	0.0 NIA	0.0 NIA	0.0 NIA	0.0 NIA	0.0 NIA	15 Moderate
	SD	0.0	0.0	0.0	0.0	0.0	2.7
Trichophyt onmentagrophytes	Mean	0.0 NIA	0.0 NIA	0.0 NIA	0.0 NIA	0.0 NIA	15 Moderate
	SD	0.0	0.0	0.0	0.0	0.0	2.2
							Itraconazole (10µg)
Aspergillus Niger	Mean	0.0 NIA	3.2 Weak	5.7 Weak	6.7 Weak	10.2 Moderate	18 Strong
	SD	0.0	1.0	1.2	2.7	2.1	2.0
Microsporu	Mea	0.0	5.3	7.5	8.0	10.7	19

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mcanis	n	NIA	Weak	Weak	Weak	Moderate	Strong
	SD	0.0	2.0	2.1	1.2	2.4	2.0
Penicillium notatum	Mean	0.0 NIA	6.2 Weak	8.0 Weak	8.4 Weak	13.7 Moderate	21 Strong
	SD	0.0	1.0	1.0	1.2	2.0	2.0

These are the actual measurements of the ZOI. The size of the disc is not included.

Legend: 0 mm No Inhibitory Activity (NIA)
1 - 8 mm Weak
9 – 16 mm Moderate
17 mm –Above Strong

The Fluconazole has inhibitory effects on the mycelia growth of *C. albicans, C. guilliermondii*, and *T. mentagrophytes*, with a zone of inhibition (ZOI) of 20mm, 15mm, and 15mm at a concentration of 10µg. Although the 15mm ZOI is Moderate, the effect maybe further improved by using a higher concentration which, is 20µg. On the other hand, Itraconazole is an effective agent against the other three fungi – *A. niger, M. canis*, and *P. notatum* with ZOI of 18mm, 19mm, and 21 mm respectively. The experiment made use of two different antifungal agents because not all antifungal agents have the same effect on specific fungus.

Mature Wellawel Leaf Extract

Shown in Table 4 is the zone of inhibition induced by the mature Wellawel leaf extract. Similar to the young wellawel leaf extract, the 100% mature leaf extract has Moderate antifungal activity against *C. albicans, A. niger, M. canis,* and *P. notatum* with corresponding ZOI of 16.3mm, 11.8mm, 11mm, and 14.0mm respectively. The results indicate that the extract has antifungal potential.

The effect of the mature leaf extract is better than the young leaf extract, as shown by the measurement of the ZOI produced against the test fungi.

Table 4. Mean Zones of Inhibition Induced by Different Concentrations of the Mature Leaf Extract on the Six Fungi

		Distilled water	25%	50%	75%	100%	Fluconazole (10µg)
Candida albicans	Mean (mm)	0.0 NIA	5.3 Weak	8.2 Weak	8.2 Weak	16.3 Moderate	21 Strong
	SD	0.0	1.0	2.4	2.1	2.0	2.4
Candida guilliermondii	Mean	0.0 NIA	0.0 NIA	0.0 NIA	0.0 NIA	0.0 NIA	16 Moderate
	SD	0.0	0.0	0.0	0.0	0.0	2.6
Trichophytonmentagrophytes	Mean	0.0 NIA	0.0 NIA	0.0 NIA	0.0 NIA	0.0 NIA	16 Moderate
	SD	0.0	0.0	0.0	0.0	0.0	2.0
							Itraconazol

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							e (10µg)
Aspergillus Niger	Mean	0.0 NIA	3.4 Weak	5.8 Weak	6.8 Weak	11.8 Moderate	18.5 Strong
	SD	0.0	1.0	2.1	1.7	2.2	2.4
Microsporum canis	Mean	0.0 NIA	5.8 Weak	7.8 Weak	8.2 Weak	11.0 Moderate	20 Strong
	SD	0.0	1.8	2.0	2.2	2.0	2.4
Penicillium notatum	Mean	0.0 NIA	6.5 Weak	8.2 Weak	8.4 Weak	14.0 Moderate	22 Strong
	SD	0.0	1.0	1.0	1.0	2.0	2.5

These are the actual measurements of the ZOI. Not included is the actual size of the disc.
Legend: 0 mm No Inhibitory Activity (NIA)
1 - 8 mm Weak
9 – 16 mm Moderate
17 mm –Above Strong
Wellawel Flower Extract

Table 5 shows the zone of inhibition produced by the different concentrations of the flower extract against the six fungi. Although all fungi were resistant, they still produced weak activities as manifested by their respective zones of growth inhibition. It is surprising, however, that *Trichophytonmentagrophytes* and *Candida guilliermondii* had no inhibitory activity against the flower extract. This result is consistent with the young and mature Wellawel leaf extracts. On the other hand, *Candida albicans*, though resistant, has the highest inhibitory activity among the six fungi studied. The result is congruent with that of the young and mature leaf extracts.

Table 5. Mean Zones of Inhibition Induced by Different Concentrations of the Flower Extract on the Six Fungi

		Distilled water	25%	50%	75%	100%	Fluconazole (10µg)
Candida albicans	Mean (mm)	0.0 NIA	3.8 Weak	5.8 Weak	7.8 Weak	8.4 Weak	19 Strong
	SD	0.0	1.0	2.2	2.0	2.6	2.2
Candida guilliermondii	Mean	0.0 NIA	0.0 NIA	0.0 NIA	0.0 NIA	0.0 NIA	15 Moderate
	SD	0.0	0.0	0.0	0.0	0.0	2.2
Trichophytonmentagrophytes	Mean	0.0 NIA	0.0 NIA	0.0 NIA	0.0 NIA	0.0 NIA	16 Moderate
	SD	0.0	0.0	0.0	0.0	0.0	3.2
							Itraconazole

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							(10µg)
Aspergillus niger	Mean	0.0 NIA	3.2 Weak	3.8 Weak	4.0 Weak	4.3 Weak	17 Moderate
	SD	0.0	1.0	1.5	1.0	1.0	2.3
Microsporumcanis	Mean	0.0 NIA	4.7 Weak	5.7 Weak	6.8 Weak	7.0 Weak	18 Strong
	SD	0.0	1.0	2.2	2.0	2.4	3.0
Penicillium notatum	Mean	0.0 NIA	3.0 Weak	4.2 Weak	5.0 Weak	6.3 Weak	19 Strong
	SD	0.0	1.8	2.0	2.0	2.1	3.0

These are the actual measurements of the ZOI. Not included is the actual size of the disc.
Legend: 0 mm No Inhibitory Activity (NIA)
1 - 8 mm Weak
9 – 16 mm Moderate
17 mm –Above Strong

Minimum Inhibitory Concentration (MIC) of the Wellawel Extract

To determine the lowest concentration of the extract that can inhibit the growth of the fungi, the researchers performed the MIC test.

Table 6. Minimum Inhibitory Concentration of the Wellawel Extract

Fungi	**Young Wellawel Leaf Extract**					
	100mg/mL	50mg/mL	25mg/mL	12.5mg/mL	6.25mg/mL	3.125mg/mL
Candida albicans	15.5mm	8.5mm	3.0mm	3.0mm	0.0mm	0.0mm
	Mature Wellawel Leaf Extract					
	100mg/mL	50mg/mL	25mg/mL	12.5mg/mL	6.25mg/mL	3.125mg/mL
Aspergillusniger	11.8mm	6.8mm	3.4mm	3.0mm	0.0mm	0.0mm
	Wellawel Flower Extract					
	100mg/mL	50mg/mL	25mg/mL	12.5mg/mL	6.25mg/mL	3.125mg/mL
Penicilliumnotatum	6.3mm	5.0mm	4.2mm	3.0mm	0.0mm	0.0mm

Of the six fungi, *Candida albicans, Aspergillusniger,* and *Penicilliumnotatum* were found most sensitive to the young leaf, mature leaf, and flower extract, respectively, with MIC of 12.5mg/mL and mean zones of inhibition of 3.0mm. It shows that even at a low concentration of the extract, it is enough to cause inhibitory activity. However, the action is resistant in terms of its overall effect.

Comparison of the Zones of Inhibition of the Different Concentrations of the Wellawel Extracts

Table 7 presents the comparison of the zones of inhibition of the different concentrations of the extract against the test fungi. Among the six fungal species studied, there are only four species that were positive for zones of inhibition. These are *C. albicans, A. niger, M. canis*, and

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P. notatum. The zones of inhibition of the three extracts (young leaf, mature leaf, and flower extracts) on *C. albican*s at 25%, 50%, 75%, and 100% are not statistically significant because of the tabular value of p is greater than 0.05. The same is true with *A. niger*, *M. canis*, and *P. notatum*. The result implies that the zones of inhibition of the 25% concentration of the young leaf extract, mature leaf extract, and the flower extract against *Candida albicans* are statistically the same. Similar result holds true for the 50%, 75%, and 100% concentrations, and to all the fungi tested.

However, when the researchers compared the effect of the different concentrations of the three extracts to one another (25% as against 50% as against 75% as against 100% as against positive control), there found a significant difference. This result implies that the extract with a higher concentration is more effective than the extract with a lower concentration. Furthermore, the positive control agents (Fluconazole and Itraconazole) are more effective that the rest of the extract concentrations. This result is not surprising since the positive controls are known antifungal agents and are in the pure form while the Wellawel extracts are still in the unpurified form.

Table 7. Comparison of the Mean Zones of Inhibition (in mm) Induced by the Different Concentrations of the Three Extracts Against the Fungi

	Candida albicans			***Aspergilusniger***			***Microsporumcanis***			***Penicilliumnotatum***		
	YLE	**MLE**	**FE**	**YLE**	**MLE**	**FE**	**YLE**	**MLE**	**FE**	**YLE**	**MLE**	**FE**
25%	3.0	5.3	3.8	3.2	3.4	3.2	5.3	5.8	4.7	6.2	6.5	3.0
50%	6.0	8.0	5.8	5.7	5.8	3.8	7.5	7.8	5.7	8.0	8.2	4.2
75%	8.0	8.2	7.8	6.7	6.8	4.0	8.0	8.2	6.8	8.4	8.4	5.0
100%	15.5	16.3	8.4	10.2	11.8	4.3	10.7	11.0	7.8	13.7	14.0	6.3
Fluconazole	20.0	21.0	19.0									
Itraconazole				18.0	18.5	17.0	19.9	20.0	18.0	21.0	22.0	19.0

YLE – Young Leaf Extract MLE – Mature Leaf Extract FE – Flower Extract

The antifungal action of the three Wellawel extracts may be attributed to its tannin content. Through this, phytochemical activity is demonstrated in the mature and young leaves but not in the flowers. The phenolic part of tannins is responsible for its antiseptic effect and may have been the phytochemical that is responsible for its antifungal activity. The study conducted shows that the higher the concentration of the extracts, the wider is the zone of inhibition exhibited against the fungi. According to Khan et al. (2010), the presence of flavonoids, tannins, cardiac glycosides, and reducing sugars in leaves may have inhibitory effects against selected strains of fungi. The inhibitory action can be in the form of cell membrane disruption resulting in the diffusion of cell organelles as observed by the irregular and wrinkled shape or appearance and loss in cell membrane integrity.

Moreover, the antimicrobial mechanisms of tannins by the astringent action may induce the formation of complexes with enzymes or substrates, resulting in the inhibition of the microbial enzymes in raw culture filtrates or purified forms. Draughon (2004) confirmed the antimicrobial

effect of alkaloids and tannins. Accordingly, these bioactive substances are responsible for the antimicrobial effect of the extract against the fungus. It affects the protein synthesis of the organism affecting the production of enzymes that may be beneficial to the growth and development of the fungus.

Conclusions

Based on the findings of the study, the researchers conclude the following (a) The young and mature Wellawel leaf extracts contain tannins, alkaloids, and flavonoids, while the flower extract contains alkaloids and flavonoids only; (b) Both young and mature Wellawel leaf extracts exhibited moderate inhibitory activity against *C. albicans, A. niger, M. canis,* and *P. notatum*, while both have no inhibitory activity against *T. mentagrophytes* and *C. guilliermondii*. The flower extract has either weak or no inhibitory activity against all the fungi. Both the positive control agents (Fluconazole and Itraconazole) exhibited either moderate or strong inhibitory activities against the six fungi; (c) The minimum inhibitory concentration of the Wellawel extracts was 12.5mg/mL manifesting a zone of inhibition of 3.0mm. The young leaf extract was most sensitive to *Candida albicans,* the mature leaf extract to *Aspergillusniger,* and the flower extract to *Penicilliumnotatum*; (d) The mean zones of inhibition exhibited by each concentration of the extract are statistically the same across all the six fungi tested. The zones of inhibition exhibited by each concentration of the three extracts are statistically different from each other.

Recommendations

Based on the results of the study, the researchers recommend the following: (a) to test the extract with other fungal species; (b) to employ another solvent for extraction that could effectively extract all of the active substances; (c) to formulate a pharmaceutical product like an ointment or cream made from the Wellawel leaf extract as an antifungal agent; and (d) to encourage people to cultivate Wellawel in the communities.

Acknowledgment

The researchers wish to thank the University of Northern Philippines for funding this project.

References

1. Burton, G.R.W., Engelkirk, P.G. &Duben-Engelkirk, J.L (2011). Burton's Microbiology forHealth Sciences. 9th Ed. Lippincott Williams & Wilkins. Baltimore, Maryland. Retrieved on December 15, 2019, from https://books.google.com.ph/books/.
2. Delost, M.D. (2004). Introduction to Diagnostic Microbiology: A Text and Workbook.Philippines: Elsevier (Singapore) Pte Ltd. Retrieved from https://www.goggle.com.ph
3. Draughon, F.A. (2004). Use of Botanicals as Biopreservatives in Foods. Food Technology 58 (2). Retrieved on December 2, 2019, from http://agris.fao.org/agris-search/search.do?recordID=US201300961995.
4. Kaur, G., Kaur, H., Kaur, M., Kumar, B. &Tiwari, P. (2011). Phytochemical Screening andExtraction: A Review. InternationalePharmaceuticaSciencia 1 (1). Retrieved on
5. December 10, 2019, from https://pdfs.semanticscholar. org/979e/9b8ddd64c 0251740bd8ff2f65f3c9a1b3408.pdf
6. Khan, Z.S. &Nasreen, S. (2010). Phytochemical analysis, antifungal activity, and mode of action of methanol extract from plants against pathogens. Journal of

AgriculturalTechnology 6(4): 793-805. Retrieved on December 20, 2019, from https://www researchgate.net/publication/268342768_.

7. Taesotikul, T., Panthong, A., Kanjanapothi, D., Verpoorte, R., &Scheffer, J.J. (2003). Antiinflammatory, antipyretic, and antinociceptive activities of TabernaemontanapandacaquiPoir. Journal of Ethnopharmacology 84 (1). Retrieved on December 2, 2019, from https://www.ncbi.nlm.nih.gov/pubmed/12499073
8. World Health Organization (WHO). "Traditional Medicine."http://www.who.int/topics/traditional_medicine/en/
9. Zimbo, M.J. (2003). Difco Manual of Dehydrated Culture Media and Reagents forMicrobiological and Clinical Laboratory Procedures. 9th Edition.Retrivedon December 10, 2019, from https://www. biodiversitylibrary. org/ia/difcomanualofdeh 09dige#page/7/mode/1up.