Phytochemical Screening of Wellawel *(Chromolaena odorata)*Leaves, its Antimicrobial and Coagulative Properties

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ABSTRACT

The study was conducted to perform phytochemical screening, antimicrobial assay and determine the coagulating effects of wellawel ***(Chromolaena odorata****) leaves. This study made use of the experimental research design in an actual laboratory set-up. There were four phases in the experimental study. Phase 1 included the preparation of the plant sample and extraction using distilled water and ethyl alcohol, Phase 2 is the phytochemical screening to determine he presence of alkaloids, quaternary bases or amine oxides, saponins, free fatty acids, cardiac glycosides, leucoanthocyanins, flavonoids, tannins, fats and oils and anthraquinones in the leaf extract, Phase 3 is the antimicrobial assay to determine the zones of growth inhibition produced by the wellawel leaf extract to test organisms and Phase 4 is the determination of the coagulating effects of the wellawel leaf against Swiss mice. Findings showed that wellawel (****Chromolaena odorata****) leaf extract contains alkaloids, cardiac glycosides, luecoanthocyanins, flavonoids and tannins. Wellawel leaf extract has weak antimicrobial activity against* ***Bacillus subtilis****,* ***Escherichia coli*** *and no anti-fungal activity on* ***Candida albicans****. The crude and aqueous extracts have the ability to shorten the coagulation time of blood on wounds inflicted on Swiss mice. Based on the findings, the following recommendations were drawn: a follow up study should be conducted to quantify, isolate and identify the type of alkaloids, cardiac glycosides, leucoanthocyanins, flavonoids and tannins present in the leaves of wellawel and other pharmacologic testing should be done using the wellawel leaves like tests for its analgesic property, antispasmodic, antihypertensive and antihelminthic properties.*

INTRODUCTION

The Philippines is rich in plant resources because of its luxurious vegetation of all kinds of tropical plants. Many of them are known for their usability and can be a panacea for many health problems. However, these plants have to be scientifically tested to prove their effectivity to be used for therapeutic purposes.

With the hardships in life today as manifested by the shortage of medicines in most health facilities and the exorbitant cost of medicines particularly antibiotics, it is being encouraged by the Department of health that Filipinos go back to the traditional modalities of treatment like herbal medicines, acupressure, acupuncture, etc.

In the early 90's, the Philippines seemed hopeful for the merging of western and alternative medicines. There was a burgeoning global movement towards alternative therapies and the beginnings of herbal medicinal research and

development. In 1992, during the term of Dr. Juan Flavier as Secretary of Health, a brochure of 10 medicinal plants (akapulko, ampalaya, bawang, bayabas, lagundi, niyog-niyogan, pansit-pansitan, sambong, tsaang gubat, yerba Buena) for common health problems was published and commercial production was pursued. In 1997, the traditional and Alternative Medicine Act (TAMA) was passed, providing a legitimizing boost to the alternative movement in the Philippines (Apostol, 2003).

Support for research and development (R & D) for traditional medicine is concretized by the integration of the traditional medicine program in the National Research and Development Plan; technical and financial grants for R & D activities; research information dissemination and technology transfer to concerned sectors. Among the different components of the traditional package, the Department of Science and Technology (DOST) has poured in a lot of resources in the development of herbal medicine. Access to drug, because of their high cost and inequitable distribution, is a major deterrent to health service delivery. Seventy percent of the population has not been provided with drug consumption of P360 per year, this is made bleaker with the fact that only 25% of the population accounts for 75% of the total sales. Sixty two percent of drug firms and 74% of pharmaceutical laboratories are concentrated in Metro manila. Presented with these facts and after a broad-based consultative process, the DOST – Philippine Council for Health Research and Development (PCHRD) ventured into a long term program on the development of drugs from indigenous sources (Banez, 2002).

A comprehensive and in-depth study of herbal plants should address the needs of the rural folks who use wild –crafted herbs as mainstay therapy for a variety of illnesses, in lieu of affordable and consequent intermittent and ineffective use of prescription pharmaceuticals. The study must translate into a comprehensible, practical and user-friendly compendium of information, that can guide the rural folk in their use and preparation of herbal therapies, in a manner sensitive to rural mythologies and folklore.

In the hitherlands and in the lowlands this plant abundantly grows and has been observed to have coagulating properties once applied to a bleeding wound. It is also known as the NPA plant because as claimed by those who have already used it during encounters between the government outlaws and military forces, it proved to be effective in stopping profusely bleeding wounds. It has also been claimed by many residents in Abra and Ilocos Sur that this plant has anti-hemorrhagic effects. The plant is known as wellawel and scientifically known as *Chromolaena odorata.* It is also known locally as hagonoi plant.

The plant wellawel is described as an erect shrub, 1-2 feet high, nearly smooth to heavy in texture. The leaves are ovate with lips pointed. The flowers are yellow and about 3 mm long. It bears seeds that are small, round and black. Medicinally, the seeds are used to treat cough and skin diseases prepared through decoction andcan also be used as a purgative (Tabudlo, 1996).

OBJECTIVES

The study aimed to perform phytochemical screening, antimicrobial activity and determine the coagulative effects of wellawel (*Chromolaena odorata*) leaves.

Specifically, the study sought to:

1. identify the phytochemical substances present in the leaves of wellawel
2. determine the antimicrobial effects of leaves on certain groups of microorganisms
3. determine the coagulating effects of the leaves using Swiss mice as specimens.

METHODOLOGY

This section presents the design of the study, materials and experimental procedures and the statistical treatment of data.

Design of the Study

This study made use of the experimental research design in actual laboratory se up. Four phases were included in the pursuit of this study:

Phase 1: Preparation of plant samples and extraction using distilled water and ethyl alcohol.

Phase 2: Phytochemical screening was done to determine he presence of alkaloids, quaternary bases or amine oxides, saponins, free fatty acids, cardiac glycosides, leucoanthocyanins, flavonoids, tannins, fats and oils and anthraquinones.

Phase 3. Antimicrobial Assay using the test organisms *Bacillus subtilis, Escherichia coli* and *Candida albicans.*

Phase 4: Determination of the Coagulating effects of Wellawel leaves

Procedures:

I. Preparation of the extract

Wellawel leaves were gathered in Metro Vigan. They were washed thoroughly to remove adhering dirt and air-dried at room temperature. They were divided into 4 sets. The first set of leaves were placed in a plastic bag and were submitted to UPNSRI at Diliman, Quzon City for the phytochemical screening of the said plant.

The other set was finely cut into small pieces. Thirty grams of the finely cut leaves were placed in an Erlenmeyer flask. Sufficient amount of distilled water was added to completely submerged the material The flask was stoppered and soaked for 48 hours. The, they were filtered using a Buchner funnel. The flask and plant material were rinsed with distilled water. The washings were combined with the first filtrate.

The filtrates were concentrates through evaporation process to about 10 ml. The exact volume of the concentrated extracts were

measured. Then, the extracts were stored in a tightly stoppered container inside the refrigerator.

The same procedure was done to another set of 30 grams of finely cut leaves. This time, ethyl alcohol was used as the solvent.

For the crude extract

Thirty grams of previously washed wellawel leaves were finely cut into pieces. The leaves were then grinded using a clean mortar and pestle to extract the juice. After grinding, it was filtered using a cheese cloth and filtered again using a glass funnel and a filter paper. The crude extract was stored in a tightly stoppered container inside the refrigerator.

II. Phytochemical Screening

Plant samples were submitted to NSRI, UP Diliman, Quezon City. Phytochemical screening was done using the following tests:

1. Dragendorff's and Mayer's tests were used to determine the presence or absence of alkaloids, quaternary bases and / or amine oxides
2. Froth test tested the presence or absence of saponins.
3. Sodium carbonate test determined the presence or absence of free fatty acids.
4. Keller-Kiliani test determined the presence or absence of cardiac glycosides.
5. Bate smith and Metcalf test was used to see the presence or absence of leucoanthocyanins
6. Wilstatter test determined the presence or absence of flavonoids.
7. Ferric chloride test screened for the presence or absence of tannins
8. Filter paper test indicated the presence or absence of fats and oils
9. Modified Borntrager's test determined the presence or absence of anthraquinones.

III. Antimicrobial Activity

This part of the study discussed the procedure in testing for the antibacterial activity of the leaf extract using the Kirby-Bauer Disk Diffusion Method.

A. The Microorganisms

The gram positive bacterium, *Bacillus subtilis*, gram negative bacterium, *Escherichia coli* and the fungus, *Candida albicans* were obtained from the stock culture from UP Los Banos.

B. Preparation of Nutrient Broth and Agar Media

Dissolve 5.0 gram peptone; 3.0 g beef or yeast extract; 2.5 g sodium chloride in 1000 ml distilled water. To prepare the nutrient agar medium, simply add 16.0 g of agar to a solution of nutrient broth. Sterilize both media at 121 ^{0} C for 20 minutes.

C. Preparation of Saboraud Glucose Broth and Agar Media

Dissolve 10 g neopeptone and 40 g glucose in 1000 ml distilled water.

D. Preparation of 0.5 McFarland Standard

Mix 0.5 milliliters of 0.048 M $BaCl_2$ (1.175% w/v $BaCl_2.2H_2O$) to 99.5 ml of 0.36 N H_2SO_4 (1% v/v). Distribute 5 milliliters into screw-cap tubes of the same dimension as those to be used in preparing the culture suspension. Seal the tubes tightly and store them in the dark at room temperature. Prior to use, shake the turbidity standard vigorously on a mechanical vortex mixer.

E. Preparation of Sterile Isotonic Saline-Tween 80 Solution

Weigh 0.85 gram sodium chloride and dissolve in 100 ml distilled water to prepare the isotonic saline solution. Measure 0.1 milliliter Tween 80 with a 1.0 ml serological pipette and add to the above 100 milliliter isotonic saline solution. Mix well to dissolve the Tween 80. Sterilize the isotonic saline **Tween 80 solution in an autoclave at 121 ^{0}C for 15 minutes.**

F. Preparation of Sterile Isotonic Saline Solution

Weigh 0.85 g sodium chloride and dissolve in 100 ml distilled water to prepare the isotonic saline solution.

Preparation of Inocula

A. Bacteria

A loopful of pure bacterial culture of *Bacillus subtilis* and *Escherichia coli* is inoculated into a 50 ml Mueller-Hinton broth and the suspension is incubated for 18-24 hours at 35-37 0 C. Aseptically transfer 5 ml of this culture in a screw capped test tube and match the turbidity with 0.5 Mc Farland.

B. ***Yeast Fungi***

Loopfuls of organisms from culture slants of *Candida albicans* are inoculated in 50 ml of Saboraud glucose broth medium. Incubate for 18 hours at room temperature. Shake vigorously for 1 minute and compare the concentration of the spore suspension with 0.5 McFarland standard. Take 0.5 ml of this adjusted spore suspension and add to 20 ml saline-Tween 80 solution. This yeast inoculum is used to swab the agar plates for the screening.

B. Adjusting the Turbidity of the Inocula

A. Bacteria . If the bacterial suspension does not appear to be of the same density as the Mc Farland standard, adjust the turbidity by adding sterile saline solution or culture broth and subsequently compare the resulting turbidity to the standard.

B. Fungi. If the fungal inoculum does not appear also to be of the same turbidity with Mc Farland standard, as for the filamentous types, add more of the spore scrapings or the saline-Tween 80 solution.

C. Preparation of Agar Plates

Pour approximately 15 ml of melted Nutrient Agar or Saboraud Glucose agar into dry and sterile Petri dishes. Let the medium solidify before use.

D. Seeding of Plates

A sterile cotton swab is dipped into the bacterial broth suspension or saline-Tween 80 spore suspension. Excess inoculum is removed by rotating the swab several times against the wall of the test tube above the fluid level. The

entire surface of the agar is streaked evenly in all directions. Let the swabbed plates stand for 5 minutes.

E. ***Placements of Disks***
Using sterile forceps, pick 6 mm disk and dip it into the alcoholic extract of wellawel leaves or control and lay and press it gently (to ensure maximum full contact of the disc with the agar medium) on the estimated center of one quadrant of the Petri dish. Three quadrants of the Petri dish are for the wellawel leaf extract and the fourth is for the control.

F. ***Incubation and Observation of the Plates***
Plates are inverted and incubated within 30 minutes of inoculation at 35-37^0 C for bacteria and at 27 ^{0} C for the yeasts.

Observe the results after 18-24 hours for bacteria and yeast.

E. Reading and Interpretation

Invert the plates when doing the reading. The diameter of each zone of inhibition is measured to the nearest tenth of a millilitre with a ruler or calliper. For purposes of standardization, the following interpretative range of standard zones is adapted.

Zone of Growth Inhibition (in mm)	Activity
> 17	+++ strong
12-16	++, moderate
7-11	+, weak
6 and <	-, negative

IV. Determination of the Coagulating Effect

Nine adult Swiss mice of the same weight and age were randomly chosen. These samples were divided into three groups.

Using a sterilized scalpel blade, laceration of about 1 millimeter deep and two millimetres long was inflicted below the groin along the lateral sides of both hind limbs. The coagulation time of the wound was noted and recorded. This procedure was repeated three times and the average coagulation time was computed. This serves as the control.

The same procedure was repeated to the second group of test animals. This time, the wound was treated with 1 milliliter of wellawel crude extract. The coagulation time of the wound was noted and recorded. This procedure was repeated three time and the average coagulation time was computed.

The same procedure was again repeated for the third group of test animals using the aqueous extract. (Tabudlo, 1996)

Statistical treatment of Data

The chemical qualitative analysis was employed for the detection of the presence or absence of chemical constituents under consideration.

The mean was used to describe the average coagulating effects and zones of growth inhibitions produced by the wellawel leaves.

RESULTS AND DISCUSSIONS

1. Phytochemical Screening

Results on the phytochemical screening of the wellawel leaves (*Chromolaena odorata)* are presented in Table 1.

Table 1. Results of the Phytochemical Screening of Wellawel Leaves (*Chromolaena odorata)*

Tests	***Chromolaena odorata* Leaves**	
	Results	**Indication**
1.. Dragendorff's Test	Formation of orange precipitate	Presence of alkaloids
2. Mayer's Test	Formation of white precipitate	Presence of alkaloids
3. Dragendorff's and Mayer's Tests	No reaction	Absence of Quaternary Bases and/or Amine Oxides
4. Froth test	Did not form 3 cm honeycomb froth that persisted after 30 mins.	Absence of Saponins
5. Sodium carbonate test	No reaction	Absence of Free Fatty acids
6. Keller Kiliani Test	Reddish-brown color at the interface	Presence of Cardiac glycosides
7. Bate-Smith & Metacalf test	Violet coloration	Presence of leucoanthocyanins
8. Wilstatter Test	Red coloration	Presence of Flavonoids
9. Ferric Chloride test	Brownish green precipitate	Presence of Tannins
10. Filter Paper test	No greasy appearance	Absence of Fats and Oils
11. Modified Borntrager's test	No reaction	Absence of Anthraquinones

Alkaloids. As gleaned from Table 1, C. odorata leaves yielded positive result fo alkaloids using Dragendorff's test. This isevidenced by the formation of an orange precipitate.

Further, Mayer's test confirmed presence of alkaloids with the formation of white precipitate.

Alkaloids are widely used in medicines like morphine, codeine, etc. Alkaloids are anti-hypertensive, antineoplastic agents and demonstrate encolytic property. It is used to relieve nasal congestion, stop hemorrhage, combat malaria and dilate the pupil of the eye and also used as a muscle stimulant. (The US

Educator Encyclopedia, 1984). The leaves of *Chromolaena odorata* can be a potential cure for illness related to the above mentioned diseases.

Quaternary Bases / Amine Oxides

Using Dragendorff's and Mayer's tests revealed the absence of quaternary bases and / or amine oxides in the leaves of *Chromolaena odorata.*

Saponins

The leaves of *C. odorata* do not contain saponins. On Froth test, the leaf extract did not form 3 cm honeycomb froth that persisted for 30 minutes.

Free fatty Acids

There was no reaction of the leaf extract on Sodium Carbonate test. This means that the leaves of *C. odorata* do not contain free fatty acids.

Cardiac Glycosides

The leaf extract of *C. odorata* contains cardiac glycosides. This was evidenced by the formation of a reddish brown color at the interface using the Keller –Kiliani Test. Cardiac glycosides have effects on the heart and kidneys and affect the contractions of the heart muscles.

Leucoanthocyanins

The presence of leucoanthocyanins was detected on the leaf extract of *C. odorata* as it yielded a violet coloration using the Bate-Smith and Metcalf Test. Leucoanthocyanins have been reported to improve biological properties of blood vessels leading to their use in the therapy of such different types of vascular disorders as capillary fragility and peripheral chronic venous insufficiency and microangiopathy of the retina. (www.google.com)

Flavonoids

Flavonoids can also be found in the leaf extract of wellawel as evidenced by the formation of a red color using Wilstatter test. Flavonoids have antiviral, anti-inflammatory and cytotoxic properties (Capal, 1992)

Tannins

Using Ferric chloride test, the presence of tannins was detected by the formation of a brownish-green precipitate. Tannins are possible sources of chemicals for the treatment of diarrhea and extensive burns and maybe used rectally for the relief of various rectal disorders (Santos, 1985). It is also used n the treatment of bed sore and weeping ulcers. It was formerly used for sore throat and stomatitis.

Fats and Oils

C. odorata leaves do not contain fats and oils as there was no greasy appearance on Filter Paper test.

Anthraquinones

Anthraquinones were also found to be absent in the leaves of wellawel. The leaf extract gave no reaction using the Modiefied Borntrager's Test.

2. Antimicrobial Assay

Table 2 presents the zones of growth inhibitions on the test organisms using wellawel leaf exract (*Chromolaena odorata)*

Table 2. Zones of growth Inhibition (in mm) of the Test Organisms Using Wellawel Leaf Extract (*Chromolaena odorata)*

Test organisms	Trials			Mean	Antimicrobial Activity
	1	2	3		
Bacillus. Subtilis	7.03	8.71	8.73	8.15	Weak
Escherichia coli	9.73	9.73	8.71	9.39	Weak
Candida albicans	*6.0*	6.0	6.0	6.0	Negative

Legend:		
	> 17	+++ strong
	12-16	++, moderate
	7-11	+, weak
	6 and <	-, negative

It can be shown in Table 2 that the ethanolic extract of wellawel leaves produced weak inhibitions against the growth of *B. subtilis* (x = 8.15) and *E. coli* (x=9.39) and no effect on the growth of *C. albicans* (x=6.0)

The weak antimicrobial activity of wellawel leaves can be attributed to the presence of alkaloids and flavonoids in the extract, since plants containing these phytochemicals are excellent antibacterial agents.

In a similar study conducted by Arce and Barroga (2007) they also performed antimicrobial activity using the stem of wellawel plant and found out that there is a moderate antimicrobial activity of stem extract against C. albicans and weak antimicrobial activity against S. aureus and P. aeruginosa. Their results differed in the sense that the leaf extract showed no antifungal activity while the stem extract showed moderate antimicrobial activity. Both of the stem and leaf extract showed weak antimicrobial activity against gram + and gram – types of microorganisms.

Another difference in this study and that of the study of Arce is that they performed 3 replicates each having 4 quadrants for each microorganism while this study made use of one replicate with also 4 quadrant for each microorganism.

3.Coagulative Effects of *C. odorata* leaves

Table 3 reveals the coagulative effects of wellawel leaves (*Chromolaena odorata*) to the wounds artificially inflicted to Swiss mice.

Table 3. Coagulating Time (in mins) of Swiss Mice Using Wellawel (*Chromolaena odorata*) Leaf Extract

Treatments	Trial 1	Trial 2	Trial 3	Average
Control	1.33	1.22	1.36	1.30
Crude Extract	0.86	0.80	0.85	0.84
Aqueous Extract	1.24	1.31	1.25	1.27

Table 3 reveals the results of the coagulativ effects of *Chromolaena odorata* leaves.

For the control, in which no wellawel extract was used, the incised wound took an average time of 1.30 minutes to stop bleeding. The Swiss mice whose wounds were treated with 1 ml of crude extract of wellawel leaves have an average coagulation time of 0.84 minutes. The addition of 1 ml of aqueous extract of wellawel leaves stopped the bleeding of the incised wound at 1.27 minutes..

The results imply that application of wellawel leaf extracts to wound can shorten bleeding time as compared to the wound with no treatment of the leaf extract. In humans, the normal coagulation time takes bout 2-4 minutes. (Sirridge, 1990)

Comparing the two wellawel extracts, the crude extract gave a shorter coagulation time (0.84 minutes) than the aqueous extract (1.27 minutes).

The ability of the wellawel leaf (*Chromolaena odorata*) extract to shorten the blood coagulation can be attributed to the presence of tannins in the leaves which are known for their ability to precipitate proteins. Plants containing tannins are known to possess astringent properties and can be used in the treatment of minor ulcerations.

Another reasons why wellawel have coagulating effects is the presence of leucoanthocyanins. These chemical substances have been reported to improve biological properties of blood vessels . (www. Google.com)

The results are similar with ones obtained in the study of Tabudlo (1996) that hagonoi leaf and stem extracts have coagulatory effects when applied to the artificial wounds of male Swiss mice.

CONCLUSIONS

Wellawel leaf (*Chromolaena odorata)* extracts contains alkaloids, cardiac glycosides, leucoanthocyanins, flavonoids and tannins. These substances can become excellent sources of therapeutic substances.

Wellawel leaf extract has weak antimicrobial activity against *Bacillus subtilis*, *Escherichia coli* and no fungal activity against *Candida albicans.*

The crude and aqueous extracts has the ability to shorten the coagulation time of blood on inflicted wounds using Swiss mice.

RECOMMENDATIONS

1. A follow up study should be conducted to quantify, isolate and identify the type of alkaloids, glycosides, lucoanthocyanins, flavonoids and tannins present in the leaves of wellawel.

2. Other pharmacological testing should be done using the wellawel leaves and other parts of the plant like test for its analgesic property, anti-spasmodic, anti-helminthic and anti-hypertensive properties.

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