Phytochemical Screening of Wellawel *(Chromolaena odorata)* Leaf Extract

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Abstract

The study was conducted to perform phytochemical screening and antimicrobial assay in order to 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 was the phytochemical screening to determine the presence of alkaloids, quaternary bases or amine oxides, saponins, free fatty acids, cardiac glycosides, leucoanthocyains, flavonoids, tannins, fats and oils and anthraquinones in the leaf extract, Phase 3 was the antimicrobial assay to determine the zones of growth inhibition produced by the wellawel leaf extract to test organisms and Phase 4 was 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 and Escherichia coli; it has no anti-fungal activity on Candida alhicans.*

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. Other pharmacologic testings should be done using the wellawel leaves like tests for its analgesic property, antispasmodic, antihypertensive, and antihelminthic properties.

Introduction

Background of the Study

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 I0 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. I 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 folks 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. It has been observed that this plants has 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 wellawel plant is described as an erect shrub, 1-2 feet high, nearly smooth to heavy in texture. The leaves are ovate with tips pointed. The flowers are yellow and about 3 mm long. It hears seeds that are small, round and black. Medicinally, the seeds are used to treat cough and skin diseases prepared through decoction and can also be used as a purgative (Tabudlo, 1996).

Objectives

The study aimed to perform phytochemical screening and antimicrobial assay to 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; and
3. determine the coagulating effects of the leaves using Swiss mice as specimens.

Scope and Delimitation

The study was delimited to the phytochemical screening and the determination of the anti-microbial effects of wellawel leaves as well as its coagulating effects.

Only the leaves were used in the experimental investigation. Leaves of wellawel were gathered mainly from Metro Vigan.

The determination of the chemical constituents was limited to the qualitative rather than the quantitative analysis. Only the presence or absence of a general group of organic

compounds like alkaloids, quaternary bases or amine oxides, saponins, free fatty acids, cardiac glycosides, leucoanthocyanins, flavonoids, tannins, fats, and anthraquinones were given focus.

Swiss mice were used as subjects in the determination of the coagulating properties of the leaves of wellawel.

The antimicrobial activity was limited to the determination of the growth of zones of inhibitions produced by a relative group of microorganisms against the leaf extract. *Bacillus subtilis* was the representative bacteria for gram positive bacteria, *Escherichia coli* represented the gram negative bacteria while *Candida albicans* represented the fungal group of bacteria.

Plant samples were submitted to the NSRI, UP Dilirnan, Quezon City for phytochemical screening. The antimicrobial assay and the coagulating effects of the leaves were done at the Biotech Laboratory and Research Laboratory of UNP, Vigan, Ilocos Sur.

Review of Related Literature

This section includes **a** summary of readings and studies of both local and foreign researches which are relevant to the study.

Modern pharmaceutical drugs are typically based on single chemicals with "active" properties. Many drugs have been discovered through research into the physiological effects of chemicals found in plants. Some of these drugs are still derived directly from plants (e.g. digitalin from foxglove, *Digitalis)* while others are now synthesized (e.g. aspirin inspired by the medicinal properties of the bark of the willow, *Salix alba).* Chemicals in some plants are extracted and transformed, providing the building blocks of drugs, for example progesterone synthesized from chemicals found in some species of *Dioscorea,* the wild yam (BGCI Fact Sheet, 2000).

Guevara and Recio as cited by Banez. (2002) mentioned that there has been in more recent times an awakening towards the use of drugs and their preparation in a kind of "back-to-nature" movement instead of the classical synthetic compounds manufactured in advanced countries. While the use of synthetic drugs is of undoubted value especially in advanced state of illnesses, it is believed that the use of herbal medicines of properly tested efficacy would be of great advantage in a developing country like the Philippines which is blessed with bountiful plant resources. The idea is to keep people healthy by treating illnesses at an early stage instead of resorting to treatment when the disease is already at an advanced stage.

The importance of herbal plants offer alternative remedies with tremendous opportunities. They do not only provide access and affordable medicine to poor people. They can also generate income, employment and foreign exchange for developing countries. People who live in rural areas of the Asia Pacific are familiar with the medicinal properties of plants, growing close to their homes, in the open fields, water margins, waste lands, both inside and outside the nearby forest areas and under different growth conditions. Most of the plant materials collected used are fresh, either to obtain the extract from the whole plant or parts thereof, whether they be leaves, roots, flowers or fruits. In case of woody forms, mostly the bark, roots and other parts are used. Carminatives like ginger, cloves and coriander are also usually added as fresh or dried materials. Though dried plant parts are frequently used, often the easy availability of fresh material is a critical point and the herbal doctor in the village is well familiar with various plants he needs, their growth patterns, seasonality, habitat and other details. Such details were usually passed on in the past from parent to offspring in the family and uses of plants and the various combinations or mixes made were kept as a family secret. Along with the development of knowledge at family level, tremendous progress has been made at using the plant products at professional level in different societies, which have grown into branches of science in their own right. Most of the methods and uses were taught orally and through demonstration and very few records or writings were maintained. Such professional practices are continuing even today, As villagers migrated to the city, losing touch with past practices or when there was no heir apparent to the village doctor, the precious knowledge is usually lost (Amanonce et.al., 2007).

Traditional medicine has to be considered as the cost of health care has become unaffordable to a great majority of the population. A big segment of the population believe in traditional cures, yet we have not documented its effectiveness. In the Region, we should first come up with the profile of the different modalities of treatment and determine their acceptability. Further, there is a need to define traditional medicine (Cayabyab, 2004).

Funded programs of the Department of Science and Technology are aimed at intensify research on indigenous plant materials not only for drug manufacturers but also primarily for providing the rural areas with adequate supply of medicines or drug preparations by the expanded utilization of the plants in their raw and semi-processed forms.

Primary screening provides a general profile of the toxicity, pharmacological activities, and pharmacokinetics of a new drug. The results obtained with the animal models are used to evaluate the safety of the material, its toxic effects, and its intended therapeutic properties. Thus, it is essential that the pharmacological and toxicological properties of the drug material be established before any clinical trials on man are conducted.

Tabudlo (1996) conducted phytochemical analysis on the leaves and stems of the hagonoi plant *(Chromolaena odorata).* It was found that the aqueous and alcoholic leaf extracts of hagonoi plant contain glycosides, saponins, and tannins. Sterols were extracted only from the aqueous medium while terpenes were only extracted from the alcoholic medium. Further, the study also found out that there are coagulating effects of the aqueous and alcoholic leaf and stem extracts of the hagonoi plant using male Swiss mice as test animals.

This study is different from Tabudlo's scientific investigation in that, though both studies utilized the same plant, this study concentrated more on the leaves of wellawel. Morever, antimicrobial assay was also conducted to determine if the leaf extract has an inhibitory effect towards the growth of certain types of microorganisms. Crude extract and aqueous extract of the leaves were also used to test the coagulatory effects of the leaves on Swiss mice.

Viado (2006) performed phytochemical, mirobiological screening and pharmacological testing on water hyacinth *(Eichornia crassipes* Linn) and kataka-taka *(Kalanchoe pinnata* Linn). Results of the phytochemical screening indicated that the water hyacinth contained flavonoids, tannins, and resins in the leaves and the kataka-taka leaves contained alkaloids, saponins, flavonoids, and resins. The Kirby Bauer Disk Diffusion method showed that the kataka-taka crude leaf extract possesses an active inhibitory activity against *Staphylococcus aureus* and partial inhibitory activity against *Escherichia coli* and *Candida albicans.* The water hyacinth did not manifest inhibitory activity against bacteria and fungi test organisms.

The water hyacinth and kataka-taka also showed a positive coagulating effect as shown by the 48 seconds and 82.75 seconds coagulation time compared to the 82.75 seconds coagulating time of the positive control group and 93.25 seconds coagulation time of the negative control group.

This study is basically similar with Viado in that both studies performed phytochemical, microbiological screening and tested the coagulating properties of the said plant. They only differ in the plant specie used.

Rabena (1999) conducted the isolation, characterization and identification of the active components of kakawate leaves *(Gliricidia sepium Jacq. Kinth.Ex.Walph)* against termites. Chopped kakawate *(Gliricidia sepium Jacq.Kinth. Ex. Walph)* were soaked in petroleum ether. Crude extract recovery corresponded to 5.35% of the total fresh weight of the leaves. Feeding the termites with petroleum ether crude extracts at 0.02 g/ml gave 100% mortality rate within four hours and 100% within 8 hours. One hundred termite mortality is observed four hours after feeding 0.02 g/ ml (20,000 ppm) purified coumarin. Thin layer chromatography of the petroleum ether crude extract gave two major fractions.

Chromatography on a column packed with silica gel 60 and elution 4:6 petroleum ether; chloroform yielded a pure compound, CH6O2, which accounted for 2.83% of the leaf fresh weight. It was characterized by UV, IR, MS, 1H NMR and 13C NMR.

Operational Definition of Terms

Alkaloids. These are chemical substances which are nitrogen heterocycles which occur mainly in plants as the sails of common carboxylic acids. They constitute an indispensable and most potent group of substances for the treatment and mitigation of functional disturbances and relief from suffering. Alkaloids are anti-hypertensive, antineoplastic agents and demonstrate anti-tumor activity.

Amine oxides. They are also known as amine-N-oxide. They are oxides of tertiary amines including nitrogen containing aromatic compounds like pyridine. They are common metabolites of medication and psychoactive drugs and anti-cancer drugs.

Antimicrobial activity. This is referred as a test used to determine the resistance or sensitivity of antimicrobial agents against bacterial pathogens.

Antimicrobial agents. As used in this study, these are the extracts coming from wellawel leaves. Resistance or sensitivity is indicated by the presence or absence of a zone of inhibition.

Anthraquinones. These refer to the aromatic organic compounds derivative of anthracene with **a** formula of CHO. They naturally occur in plants, fungi, lichens and insects where they serve as basic skeletons for their pigments. Anthraquinone derivatives tend to have laxative effects.

Cardiac glycosides. These refer to the chemical group of glycosides used to treat heart failure and irregular heart beat . An example is digitoxin.

Coagulative effect. This refers to the activity of the extract to shorten blood coagulation time or solidify the blood form he inflicted wounds of the test animals. This is also referred to as anti-hemorrhagic property.

Control (Animal). This refers to the group of test animals which were not given wellawel extract upon inflicting with wounds.

Control (Extract). This refers to the ethyl alcohol used in the antimicrobial activity of the leaf extract.

. **Extract.** This refers to the solution obtained from the leaves of wellawel plant. In this study, there were three kinds of extracts that were used namely: ethanolic, aqueous, and crude extract.

Ethanolic extract. This refers to the solution obtained from the leaves of wellawel plant using ethyl alcohol as the solvent.

Aqueous extract. This refers to the solution obtained from the leaves of wellawel plant using distilled water as the solvent.

Crude extract. This refers to the solution obtained from the leaves of wellawel plant after it has been pounded and filtered with no solvent used.

Flavonoids. These are naturally occurring phenolic compound belonging to a large group that includes many plant pigments. Flavonoids have beneficial effects in the human diet as antioxidants, neutralizing free radicals which damage body tissues and leads to heart disease, stroke, and cancer.

Free Fatty **Acids.** These are carboxylic acids with long unbranched aliphatic tails which are either saturated or unsaturated. They are produced by the hydrolysis of the ester linkages in fats or biological oils with the removal of glycerol.

Fats/Oil. It consists of wide groups of compounds that are generally soluble in organic solvents and largely insoluble in water. Oil is used to refer to fats that are liquid at normal room temperature.

Leucoanthocyanins. They are natural polyphenols belonging to the class of bioflavonoids.

Phytochemical Screening. It is a series of tests that determine the presence or absence of certain chemical substances present in a plant The chemical considered in this study are alkaloids, quaternary bases/amine oxides, saponins, free fatty acids, cardiac glycosides, leucoanthocyanins, flavonoids, tannins, fats and oils and anthraquinones.

Saponins. These are glycosides with distinctive foaming characteristics. They consist polycyclic glycone that is either a choline steroid or triterpenoid attached via C3 and ether bound to a sugar side-chain.

Tannins. They are substances which occur as mixture of polyphenols which are very difficult to separate since they do not crystallize.

Test animals. These refer to the Swiss mice used to test the coagulating effects of wellawel.

Test organisms. These refer to the pure culture of microorganisms used in the antimicrobial assay. In this study, the microorganisms used are *Bacillus subtilis, Escherichia coli.* and *Candida albicans*.

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 set 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 the 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 din and air-dried at room temperature. They were divided into four sets. The first set of leaves were placed in a plastic bag and were submitted to UPNSRI at Dilirnan, Quezon City for the phytochemical screening of the said plant.

The other set of leaves were 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 submerge the material. The flask was stoppered and soaked for 48

hours. Then 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 concentrated 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, ground using a clean mortar and pestle to extract the juice. After grinding, it was filtered using a cheese cloth. And it was 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. Dragendorffs and Mayer's tests were used to determine the presence or absence of alkaloids, quatemary 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; and
9. Modified Bontrager's test determined the presence or absence of anthraquinones.

m. 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 subilis,* 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

Five gram peptone, 3.0g beef or yeast extract and 2.5 g sodium chloride were dissolved in 1000 ml distilled water. To prepare the nutrient agar medium, 16 g of agar was added to a solution of nutrient broth. Both media were sterilized at 121C for 20 minutes.

C. Preparation of Saboraud Glucose Broth and Agar Media

Ten grams neopeptone and 40g glucose were dissolved in 1000 m distilled water.

D. Preparation of 0.5 McFarland Standard

0.5 milliliters of 0.048 M BaCl (1.175% w/v BaCl.2HO) was mixed to 99.5 ml of 0.36 N H,SO, (1% v/v). Five milliliters was distributed into screw-cap tubes of the same dimension as those to be used in preparing the culture suspension. The tubes were sealed tightly and stored in the dark at room temperature. Prior to use, the turbidity was shaken vigorously on a mechanical vortex mixer.

E. Preparation of Sterile Isotonic Saline-Tween 80 Solution

0.85 gram sodium chloride was weighed and dissolved in 100 ml distilled water to prepare the isotonic saline solution. 0.1 milliliter Tween 80 was measured with a 1.0 ml serological pipette and added to the above 100 milliliter isotonic saline solution. The solution was mixed to dissolved the Tween 80. The isotonic saline Tween 80 solution was sterilized in an autoclave at 121"c for 15 minutes.

F. Preparation of Sterile Isotonic Saline Solution

0.85g of sodium chloride was weighed and dissolved in 100 ml distilled water to prepare the isotonic saline solution.

Preparation of]ocula

A, Bacteria

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

B. Yeast Fungi

Loopfuls of organisms from culture slants of *Candida albicans* were inoculated in 50 ml of Saboraud glucose broth medium. The culture slants were incubated for I8 hours at room temperature. The culture slants were shaken vigorously for 1 minute and was compared to the concentration of the spore suspension with 0.5 McFarland standard. 0.5 ml of the adjusted spore suspension was added 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, the turbidity was adjusted 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 also appear to be of tbe same turbidity with Mc Farland standard, as for the filamentous types, more scrapings or the saline-Tween 80 solution was added to the fungal inoculum.

C, Preparation of Agar Plates

15 ml of melted Nutrient Agar or Saboraud Glucose agar was poured into dry and sterile Petri dishes. The medium was solidified before use.

D. Seeding of Plates

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

entire surface of the agar was streaked evenly in all directions. The swabbed plates were allowed to stand for 5 minutes.

E. Placements of Disks

With the use of sterile forceps, 6mm disks were picked and dipped into the alcoholic extract of wellawel leaves or control were laid and pressed 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 were for the wellawel leaf extract and the fourth was for the control.

F. Incubation and Observation of the Plates

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

Results were observed after 18-24 hours for bacteria and yeast.

E. Reading and Interpretation

The plates were inverted when the reading was done. The diameter of each zone of inhibition was measured to the nearest tenth of a millilitre with a ruler or calliper. For purposes of standardization, the following interpretative range of standard zones was 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

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 Wellael 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 for alkaloids using Dragendorff's test. This was evidenced by the formation of an orange precipitate.

Further, Mayer's test confirmed the 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 haemorrhage, 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 Dragendorffs and Mayer's tests revealed the absence of quaternary bases and/or amine oxides in the leaves of *Chromolaena odorata* was revealed.

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 fonnation 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 fonnation 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 fonnation of a brownish-green precipitate.

Tannins are possible sources of chemicals for the treatment of diarrhea and extensive bums and maybe used rectally for the relief of various rectal disorders (Santos, 1985). It is also used in 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 the 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.

Antimicrobil 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
	I	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 on 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 the wellawel plant and found out that there was 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 the stem and the leaf extract showed weak antimicrobial activity against gram + and gram – types of microorganisms.

Another difference of this study and that of the study of Arce and Barroga is that the latter performed three replicates each having four quadrants for each microorganism; while the former made use of one replicate with also four quadrants for each microorganism.

Coagulative Effects of *C. odorata* leaves

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

Table 3. Coagulating Time (in ruins) of Swiss Mice Using Wellawel *(Chromolaena odor@ta)* 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 coagulative 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 wounds can shorten bleeding time as compared to the wounds with no treatment of the leaf extract. In humans, the normal coagulation time takes bout 2-4 minutes. (Siridge, 1990)

Comparing the two wellawel extracts, the crude extract gave a shorter coagulation time (0.84 minutes) shorter 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 reason for the presence of coagulating effects in the wellawel extract 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 the 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.

Conclusion

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 has no fungal activity against *Candida albicans.*

The crude and aqueous extract have 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 phannacological testing should be done using the wellawel leaves and other parts of the plant like tests for its analgesic property, anti-spasmodic, anti-helminthic, and anti-hypertensive properties.

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