A Metacognitive Experimatation on the Spectrum of Antibacterial Activity of the Centella Asiatica Extracts

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Abstract

This study sought to determine the spectrum of antibacterial activity of the extracts of centella asiatica extracts. The findings from the antibacterial test indicated that all the solvents employed in extracting the antibacterial elements from C. asiatica successfully impeded the growth of S. aureus and E.coli. Nevertheless, solely the undiluted extracts obtained from each solvent exhibited a broader transparent region where bacterial growth was inhibited. Additionally, the outcomes revealed that distilled water proved to be the most efficient solvent in extracting the antibacterial component of Centella asiatica when evaluated against E.coli. From a statistical standpoint, the results demonstrated that there was no significant difference in the antibacterial efficacy of the habitats being investigated.

Keywords: ***metaognitive experimentation, spectrum, antibacterial activity, centella asiatica, extracts***

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Introduction

The Philippines possesses a rich abundance of plant species that have long been recognized for their medicinal properties and historical use in healing (Asis, 1996). In recent times, there has been a growing global interest in the field of medicinal plants, with many nations now recognizing not only their potential in addressing health issues, but also their economic significance.The current resurgence and accelerated interest in herbal medicine in the Philippines are particularly noteworthy due to the country's abundant botanical heritage. These plants have proven to be valuable remedies for a significant portion of the population, especially in rural areas where access to conventional medicines is limited. However, there is a dearth of accessible information about these plants (Ladion, 1985). While significant efforts have been made to address public health issues, they are not nearly sufficient to meet the needs of a rapidly growing population.

The ability of plants to efficiently transport both organic and inorganic nutrients, including water, throughout their structures plays a crucial role in determining the overall form, function, and development of the plant as a whole, as well as its individual components (Raven, et al., 1986).Land plants face a diverse range of challenges. They must contend with periodic droughts and rapid fluctuations in temperature, both on a daily and seasonal basis, in most regions. They must also withstand unfavorable growing conditions during certain seasons and often encounter mineral-deficient substrates.

Additionally, the gravitational force impacts land plants more significantly compared to aquatic plants (Raven, et al., 1986).Thus, plants require favorable ecological conditions in order to thrive, but often they must adapt to the resources available in their environment (Mühlberg, 1982).

Gotu Kola, specifically the Centella Asiatica variety, is a slender and creeping plant found predominantly in Madagascar, with other varieties also present in India and neighboring countries. The form and appearance of Gotu Kola can vary significantly depending on the surrounding environment. In shallow waters, it develops floating leaves, while in arid locations, the leaves become small and thin with numerous roots (http://www.nutriniart.com, July, 2000). Ensuring a reliable source of high-quality Gotu Kola requires continuous effort, as it often grows in marshy areas and unintentional contamination with dirt during harvesting is common. Some areas where it grows are unsanitary, with raw sewage flowing in exposed ditches, posing a potential risk of contamination for the stands of Gotu Kola in low-lying regions. Samples from such sources have been found to contain high levels of coliform bacteria (indicative of fecal matter), mold, and yeast (www.drugstore.com). Therefore, it is essential to consider cleaner sources of Gotu Kola.

Gotu Kola, Centella Asiatica, has been widely utilized in traditional Eastern healthcare systems for various conditions (http://www.nutrimart.com/gotukola.htm).
Studies conducted in foreign countries have revealed its significant healing effects on skin, connective tissues, lymph tissue, blood vessels, and mucous membranes. Several glycosides present in Gotu Kola have been identified to possess wound healing and anti-inflammatory properties (http www.gotu.htm). However, no reports or scientific studies have investigated the constituents and antibacterial properties of the plant, particularly those derived from different habitats. Although most studies on Gotu Kola have been conducted abroad, there is currently no research available on the local variety. Hence, the researcher is motivated to examine the phytochemical content and antibacterial properties of Gotu Kola grown in both dry and watery areas abundant in Zamboanga City.

Review Of Related Literature and Studies
Plants possess a wide range of beneficial chemicals that provide various advantages to humans. These plant-based foods, aside from supplying vitamins, minerals, and fiber, also contain phytochemicals, also known as naturally occurring plant chemicals. The term "phyto" originates from the Greek word for "plant." These phytochemicals play a role in promoting well-being and reducing the risk of numerous diseases. Scientists and doctors continue to discover an increasing array of previously unknown plant compounds with complex names, such as lignins in flax, phenolics in wine and grapes, saponins in oats and alfalfa, as well as anthocyanins, lycopenes, xanthenes, isothiocyanates, and sulphoraphane.

In the year 1800, scientists specializing in chemistry and plant biology conducted investigations on plants and provided evidence that specific chemical elements were taken up from the surrounding environment. However, there was disagreement among experts regarding whether these absorbed elements were contaminants or vital components necessary for essential biological processes. Inorganic nutrients, being fundamental for basic needs and involved in crucial processes, have a significant impact on various structures and functions within the plant body when deficiencies occur.Within a specific dryland habitat, plants often exhibit different growth habits, as observed by Asis in 1971. For instance, in an abandoned patch, herbs, vines, and shrubs may coexist. Due to the distinct environmental conditions in such habitats, plants respond differently to these conditions and the factors surrounding them. Plants must acquire specific raw materials from their

environment to support the complex biochemical reactions essential for cell maintenance and growth. Plant nutrition encompasses the uptake of all necessary raw materials from the environment, the distribution of these materials within the plant, and their utilization in metabolism and growth.

Metabolism is an inherent characteristic found in all living organisms. It encompasses the processes of assimilating, transforming, and eliminating both organic and inorganic substances, accompanied by changes in energy. Heterotrophic organisms, such as animals, rely on preexisting organic compounds for nutrition. In contrast, plants are autotrophic, capable of producing their own food by converting inorganic substances into organic ones through synthesis (Mühlberg, 1982).

The natural world is remarkable in its provision of everything essential for our needs. Many conditions and illnesses can be alleviated through the utilization of plants. The plant kingdom harbors numerous undiscovered healing properties. Plant remedies are often more compatible with the human body than chemical products, making them superior agents for disease treatment. Plants possess health-promoting properties and offer advantages over chemical and inorganic medicines, as they do not produce undesirable side effects (Gerolaga, 1995).

Dayrit et al. (1987) conducted an investigation on the phytochemical constituents of Vitex Legundo L., commonly known as Lagundi. The leaves of this plant yielded four flavonoids (casticin, chrysophenol D, luteolin, and isoorientin, which are prevalent plant acids) as well as a sugar (D-fructose), which were extracted and isolated.Primitive tribes across different regions of the world have long recognized that consuming or chewing certain plants' leaves, roots, or bark can induce physiological effects. The effects vary depending on the plant, with some, like opium, being addictive drugs, while others, such as the leaves of the belladonna plant, are highly poisonous.

Alkaloids are particularly abundant in higher plants, insects, amphibians, and fungi, but much less common in mammals. Examples of alkaloids include cocaine, nicotine, mescaline, and strychnine. Most alkaloids possess some form of biological activity, and many have been harnessed for their pharmacological properties (Mann, 1994).Alkaloids primarily consist of high molecular weight compounds that are typically solid and readily crystallize, although some can be in liquid form. They generally exhibit poor solubility in water. Alkaloids are found in various plant organs (such as roots, leaves, stems, flowers, and fruits) and tissues (Doby Geza, 1965).

Similar to other nitrogen-based substances, alkaloids can be precipitated by salts of heavy metals (Johanssen, Donald Alexander, 1940).Leucoanthocyanins belong to the flavonoid group of compounds. They are colorless substances that coexist in the same cells as anthocyanins. Leucoanthocyanins can be classified into water-soluble and water-insoluble forms (Doby Geza, 1965).Flavonoids constitute a widely distributed group of phenolic derivatives that are soluble in water. Many of them exhibit vibrant colors such as red, crimson, purple, and yellow. Leucoanthocyanins are sometimes referred to as proanthocyanins because they can easily convert into anthocyanins when heated in the presence of acid.

Anthocyanins play a major role in determining the colors of numerous edible fruits. Variations in color among different fruit varieties, such as "black" and "white" cherries, are generally due to quantitative differences in the pigments present rather than changes in their nature.Tannins are a class of compounds present in specific plant species that possess the ability to transform animal hides into leather through a process called tanning. The term "tan" originates from a Latin form of a Celtic word linked to oak, as extracts from oak bark are commonly utilized as tanning agents. Tannins can be categorized into two primary groups: hydrolysable tannins

and condensed tannins, also known as non-hydrolysable tannins. Hydrolysable tannins consist of a polyhydric alcohol core, typically glucose, which is esterified with either gallic acid to form gallotannins or hexahydroxydiphenic acid to form ollagitannins. In contrast, condensed tannins solely comprise phenols of the flavone type and are often referred to as flavolans due to their polymerization of flavans. Unlike hydrolysable tannins, condensed tannins lack sugar residues (Goodwin et al., 1983).

Currently, numerous researchers are engaged in conducting experiments on untested medicinal plants. The objective is to identify potent derivatives through the hybridization of different plant species, aiming to discover effective remedies capable of combating rapidly evolving new strains of pathogens.Antiseptics refer to agents that have the ability to destroy or hinder the growth of microorganisms when applied to living tissue. They are utilized to eliminate disease-causing and other organisms present on the surface of the human body or within externally accessible body cavities (Collier's Encyclopedia).

A study conducted by Bojo et al. in 1994 examined the antibacterial activity of extracts from Peperomia pellucida, commonly known as pansit-pansitan. The findings exhibited notable antimicrobial properties against both Gram-negative bacteria (Escherichia coli, Pseudomonas aeruginosa) and Gram-positive bacteria (Staphylococcus aureus, Bacillus subtilis). The research indicated a pronounced susceptibility of Gram-negative bacteria towards the tested extracts.
In 1996, Rubith Laguna investigated the in vitro inhibitory effect of Bixa orellana (commonly known as "achuete") seed extract against S. aureus and Streptococcus pyogens. Four concentrations (25%, 50%, 75%, and 100%) in 80% alcohol were evaluated. The observed zones of inhibition were contrasted with those generated by commonly prescribed medications, specifically cloxacillin for S. aureus and penicillin for S. pyrogens. The analysis of variance (ANOVA) indicated that the B. orellana seed extract did not produce a significant inhibitory effect on the test organisms. Additionally, as the concentration of the seed extract increased, the zones of inhibition for both organisms decreased.

In medical practice, microbial cultures are isolated from patients with diseases to confirm diagnoses and assist in treatment decisions. Determining the susceptibility of microbial isolates to antimicrobial agents is a crucial task for clinical microbiologists (Madigan et al., 1997).Regulations set by the Food and Drug Administration (FDA) now govern sensitivity testing procedures in the United States, with similar regulations in place in other countries. One commonly recommended method for sensitivity testing is the Kirby-Bauer method, named after its developers (Madigan, et al., 1997). The disk-diffusion technique is widely employed to assess the overall effectiveness of antiseptics, disinfectants, and chemotherapeutic agents. This technique is technically straightforward and considered the simplest approach due to its relative affordability, allowing for the determination of an agent's overall effectiveness against a specific organism.
Centella refers to a specific variety of Gotu Kola (Centella Asiatica Hydrocotyle Asiatica) that possesses notably high levels of asiaticosides and other triterpenes. The term "centella" exclusively applies to this particular variety, while "gotu kola" is used for all other varieties.While Gotu Kola is related to plants like carrots, parsley, dill, and fennel, it lacks their feathery leaves or umbels of tiny flowers. Instead, it grows as a creeping stem in marshy areas, producing fan-shaped leaves about the size of an old British penny. Consequently, it is commonly known as Indian pennywort, marsh penny, and water pennywort.
Gotu Kola is a creeping weed that typically measures between 3 and 15 cm in length. It features stolon and rhizome-like stems. The leaves have long, erect petioles measuring 3 to 20 cm, which are usually flattened and

sheathed at their bases. The leaf blade, with a diameter of 2 to 5 cm, is palmately veined, reniform (bladder-shaped), cordate at the base, and rounded at the tip, with a scalloped margin. The inflorescence consists of simple umbels, with peduncles in pairs or groups of three, and each umbel containing 2 small bracts embracing the sessile, small, bisexual, and regular flowers, which can range in number from 2 to 5. The flowers have an inferior ovary, 5 stout stamens alternating with 5 overlapping, purplish, ovate petals. The fruit (schizocarp) is small and dry, with a roughly reticulate longitudinal ridge and contains pendulous seeds. The plant typically blooms from October to May (Asis, et al., 1971).

This herb was originally employed in India as a component of Ayurveda, the traditional system of herbal medicine, and is recognized as Brahmi, signifying "that which enhances knowledge of Supreme Reality." Within Ayurveda, it holds a highly esteemed status as one of the most invigorating herbs.The leaves of this plant that thrives in wetlands have been utilized for the management of various ailments, including leprosy, cancer, skin disorders, arthritis, and tuberculosis. It has also demonstrated efficacy in the treatment of second and third-degree burns, accelerating the healing process and minimizing the formation of scar tissue.

Gotu kola, an herb of significance in gynecology, has been effectively employed to facilitate healing following episiotomy, a surgical incision of the vulva conducted to prevent tearing during childbirth. In fact, a study published in a French medical journal in 1996 demonstrated that women treated with gotu kola after childbirth experienced swifter healing compared to those receiving standard treatments. In modern healthcare, it has found utility in conditions such as venous insufficiency, localized inflammation and infection, and post-surgical recovery.

Pharmacological investigations have revealed that gotu kola exhibits a diverse range of effects on cells and tissues involved in the process of healing, with a particular focus on connective tissues. One of its constituents, asiaticoside, plays a role in stimulating the regeneration of skin and enhancing the strength of various tissues such as skin, hair, nails, and connective tissue (source: http://www.gotu.htm).Further research has demonstrated that the individual components of gotu kola, when applied topically to wounds in laboratory rats, promote the development of healthy new connective skin tissue, increase the tensile strength of the affected area, and reduce the size of the wound. In experiments involving injections or direct implantation of asiaticoside, a constituent of gotu kola, into mice, rats, guinea pigs, and rabbits, significant effects were observed. These effects included a rapid thickening of the skin, an enhanced production of white blood cells, increased growth of new blood vessels in the connective tissue, and accelerated growth of hair and nails (file:///c MyDocument/kkk.htm).

According to Shipard (1990), gotu kola can be consumed directly from the plant, incorporated into salads, or finely chopped as a garnish for meals, similar to parsley. It possesses a mild bitter taste. Additionally, when finely chopped and added to salads or mashed potatoes, even young children are unlikely to object. The leaves can also be used fresh or dried for making tea, which can be sweetened with honey.

Methodology

A. Collection of Plant Sample

The phytochemical and antibacterial screening involved the careful selection of fresh, young, and mature leaves of Gotu Kola. The leaves were handpicked, specifically in the morning when they were still fresh and had turgid cells. Only healthy-looking leaves without insect bites, signs of injury, or discoloration were chosen.The collection of leaves took place in two different areas, one watery and the other dry, located at Johnston Compound, Canelar Moret, Zamboanga City. After being collected, the leaves were washed with running water and placed in separate clean plastic bags. They were then transported to the Laboratory for analysis.To

ensure accuracy, the screening procedure was replicated five times for each analysis.

B. Preparation of Plant Extract

1. **Alcohol Extraction for Phytochemical Analysis.** The leaves were finely cut and placed in clean containers. Using a digital pan balance, 500 grams of the plant material was accurately weighed.Next, 750 ml of 80% ethyl alcohol was measured in a graduated cylinder and transferred to a 1,000 mL Erlenmeyer flask. The finely cut plant material was mixed with a small amount of the 80% ethyl alcohol, gradually adding it until the entire 750 ml had been used. This mixture was allowed to stand in a beaker, covered with foil, immersed in the 80% ethyl alcohol for a duration of 48 hours.

To separate the liquid extract from the plant material, the mixture was poured while stirring through a Buchner funnel lined with filter paper, which was fitted to a suction flask. The pulp was manually squeezed to remove any excess solvent, and this excess solvent was included in the filtration process. Additional fresh portions of 80% ethyl alcohol were used to rinse the flask and plant material, and these rinsings were added to the extract. The remaining plant residue was discarded.

The resulting plant extract was concentrated to approximately 50 ml using a rotary evaporator. To determine the concentration of plant material per ml of the extract, the equivalent was calculated using the following formula:

$$\text{Plant Material Extract} = \frac{\text{Total weight of plant material}}{\text{Final Volume of Plant Extract}}$$

2. **Aqueous Extraction for Microbial Analysis.** Approximately 200 grams of plant leaves were carefully weighed and subsequently cut into small pieces. These pieces were then blended in a blender with a small amount of sterile distilled water from a measured volume of 550 mL. The resulting slurry was transferred into a 1000-mL beaker, which was covered with foil. The plant extract was allowed to stand for a duration of 24 hours, with occasional stirring.

After the designated time, the extract was filtered into a 500 mL Erlenmeyer flask. From the obtained aqueous plant extract, three different concentrations were prepared: 75%, 50%, and 25% respectively. Each concentration was covered and refrigerated until it was ready to be used.

3. **Alcohol Extraction for Microbial Analysis.** About 300 grams of plant leaves were chopped into small pieces and further pulverized using a sterile blender. The pulverized leaves were then placed in an Erlenmeyer flask, and enough 95% ethyl alcohol was added to completely immerse the leaves. The flask was covered and allowed to soak for 48 hours. The leaves were filtered using a Buchner funnel, and the flask was rinsed with fresh portions of alcohol. The combined washings were mixed with the filtered liquid, while the plant residue was discarded. The filtered liquid was concentrated to around 50 mL using a rotary evaporator. Next, the liquid was divided into four parts. The first part was diluted to a concentration of 75%, the second part to 50%, the third part to 25%, and the remaining part was used as the pure extract.The same extraction procedure was repeated using 70% ethyl alcohol.

C. Phytochemical Screening

1. Alkaloids Approximately 2.0 mL of plant extract was evaporated in an evaporating dish over a water bath until it reached a syrup-like consistency. While constantly stirring for five minutes, 5 mL of 2 M hydrochloric acid (HCl) was added to the concentrated extract. The mixture was then cooled to room temperature.

To the concentrated extract, around 0.5 grams of powdered sodium chloride (NaCl) were added. The mixture was stirred and filtered. Freshly prepared 2 M hydrochloric

acid was used to wash the filter paper until the filtrate reached a final volume of 5 mL.

To 3.0 mL of the filtrate, enough 28% ammonia (NH3) was gradually added to make the solution alkaline, as indicated by litmus paper. The alkaline solution was extracted three times using 10 mL portions of chloroform (CHCl3) for each extraction. The combined chloroform extracts were evaporated in an evaporating dish over a water bath.

The resulting residue was dissolved in 5 mL of 2 M hydrochloric acid, stirred over a steam bath for two minutes, and then cooled to room temperature. The solution was filtered, and the filtrate was divided into two equal parts. In one portion, a few drops of Mayer's reagent were added, and any resulting cloudiness was observed and recorded.

The result was recorded as follows:

+ = for slight turbidity

++ = for definite turbidity

+++ = for heavy precipitate

The second portion underwent the same test, with the only difference being the use of Wagner's reagent instead of Mayer's reagent. The result was recorded following the same procedure, using symbols such as (+), (++), or (+++) to indicate the outcome.In both tests, the absence of precipitate or cloudiness was interpreted as the absence of alkaloids in the plant material. A positive result for the presence of alkaloids was denoted by a recorded observation of (+), (++), or (+++).

Results And Discussion

Table 1. shows the spectrum of antibacterial activity of the extracts of C. asiatica. It revealed that as the dilution of the extracts decreases in all solvents both in dry and wet areas using S. aureus as test organism, its susceptibility decreases. S. aureus is sensitive to the pure aqueous extract and 75% aqueous extract both in dry and wet areas. It is considered sensitive to the extracts because the difference in the measurement of the zone of inhibition between the control and the extract is 3.0 mm and above. The 50% aqueous extract both in dry and wet area are considered intermediate because the difference in the measurement of the zone of inhibition between the control and the extract is 2.0-2.9 mm. Twenty five percent aqueous extract was recorded to be resistant since the difference in the measurement of the zone of inhibition between the control and the extract is 1.9 and below.

In the 95% pure alcohol extract, S. aureus was sensitive only to the extract where the plant sample was taken from the dry area having a clear zone of equal to 3 mm. The plant sample taken from the wet area was considered intermediate because the difference in the clear zone is less than 3.0 mm. The 95% alcohol extract with a dilution of 75% in both dry and wet areas were considered intermediate. S. aureus was resistant to the 95% alcohol extract with a dilution of 50% and 25% respectively.

In the two areas, dry and wet, the results are the same using 70% alcohol as solvent for extraction. The 70% pure alcohol extracts were considered sensitive; the 75% and 50% dilutions were recorded as intermediate, and the 25% dilutions were considered resistant.

Table 1. The Spectrum of Antibacterial Activity of the Extracts of Centella Asiatica

Plant Extracts	Dry Area		Wet Area	
	Test Organism		Test Organism	
	S.Aureus	E. Coli	S.Aureus	E.Coli
1. Aqueous Extract				
Pure Extract	S	R	S	R
75% Extract	S	I	S	I
50% Extract	I	S	I	S
25% Extract	R	S	R	S

2. 95% Alcohol Extract Pure Extract	S	R	I	R
75% Extract	I	R	I	R
50% Extract	R	R	R	R
25% Extract	R	R	R	R
3. 70% Alcohol Extract Pure Extract	S	R	S	R
75% Extract	I	R	I	R
50% Extract	I	R	I	R
25% Extract	R	R	R	R

Based on the average measurement of the three replicates
Legend:
S-Sensitive I-Intermediate R-Resistant

The result shows that the susceptibility of E. coli to the different solvents used to extract the antibacterial component of C. asiatica varies. As shown in Table 2, the clear zone is wider in the control than the clear zone of the extracts with different dilutions using 95% ethyl alcohol and 70% ethyl alcohol as solvents. They were considered resistant. It only shows that the wide clear zones of the extracts as concentration decreases reveal the antiseptic property of alcohol and not due to the plant extract. However, when distilled water was used as solvent for extraction, the results are more favorable. The clear zones of inhibition in the extracts with different dilutions are wider than the control. The results show that as the concentration decreases, its susceptibility increases. Pure aqueous extracts were considered resistant. The 75% aqueous extracts were considered intermediate. On the other hand, the 50% and 25% aqueous extracts were considered to be sensitive. This result simply indicates that the aqueous extracts have an inhibitory property against E. coli and that the solvent used which was distilled water was able to extract the component of C. asiatica that can inhibit the growth of E. coli. However, the result further shows that E. coli can only be inhibited in the aqueous extract with a lower concentration of the plant extract.

The difference in the sensitivity to water between S. aureus and E. coli lies in their cell wall. Generally, a gram-negative (-) bacteria like E. coli has a multilayered structure cell wall, but has a thin peptidoglycan or murein layer that is in the form of gel rather than a compact layer. On the other hand, a gram-positive (+) bacteria like S. aureus has a normally thick, homogeneous cell wall that is composed primarily of thick peptidoglycan which often contain peptide interbridges. This peptide interbridges is lacking in most gram-negative bacteria. For this reason, E. coli allows water, particularly that which is devoid of solute like sterile distilled water, to pass through easily, whereas S. aureus, because of its thick cell wall, may allow water to pass through but to a limited extent and therefore may not undergo cytolysis.

Conclusion And Recommendations

Results of the phytochemical screening showed that C. asiatica contained alkaloids, saponins, flavonoids, hydrolysable tannins and leucoanthocyanins and does not contain anthraquinones both in dry area and wet area. Statistically, the results showed that there was no significant difference in the phytochemcial contents of C. asiatica grown in dry and wet areas. Likewise, there was no significant difference in the antibacterial property of C. asiatica grown in dry and wet areas. However, there was a significant difference in the antibacterial property of C. asiatica extracts diluted to different concentrations using different solvents. It can

also be concluded that the most effective solvent in extracting the antibacterial component of C. asiatica was sterile distilled water both in dry and watery areas.

Based on the results of this study, it is recommended that elucidation of the active components of C.asiatica be done to detect other forms of phytochemicals. Likewise, it is recommended that the stem of C. asiatica be included to test for the antibiotic potential of the plant. Furthermore, it is recommended that the active metabolite be isolated to determine the component of the plant which can inhibit the growth of bacteria. It is further recommended that the activity of the active components be determined as to whether bacteriostatic or bactericidal. Also if possible, a pharmatoxicology analysis of the extracts be conducted to find out its medicinal potential. The use of other test organisms is also recommended.

Lastly it is further recommended that the results of this study be disseminated to school officials and government agencies such as the Department of Science and Technology (DOST), National Nutrition Council (NNC), Department of Social Welfare and Development (DSWD) and Department of Health (DOH) so that it can be disseminated to the students, teachers and the public in general.

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