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Gotu Kola (Centella Asiatica): Analysis of Its Phytochemical Content and Zone of Growth Inhibition

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Abstract

The study was conducted to determine the phytochemical content and antibacterial property of Gotu Kola (C. astatica) in two different habitats. Specifically the study sought to answer the following questions: Is there a difference in the phytochemical contents of C. asiatica grown in dry and watery area?: Is there a difference in the antibacterial property of C. asiatica grown in dry and watery area?: Which solvent is effective in extracting the antibacterial component of C. asiatica grown in dry and watery area? The active components of the leaves were extructed using four solvents namely: 95%, 80%, 70% ethyl alcohol and distilled water. The 80% ethyl alcohol was used in the phytochemical screening and the rest were used in the antibacterial assay and were tested against gram-positive bacteria, S. aureus and gram-negative bacteria, E. coli. The investigation of the antimicrobial potential of the extract was carried out through microbiological assay by employing the disk-diffusion method adopted from Kirby-Bauer Technique. The phytochemical screening revealed that C. asiatica contained alkaloids, saponins, flavonoids, hydrolysable tannins and leucoanthocyanins and does not contain anthraquinone. The result of the antibacterial assay showed that all solvents used in the extraction of the antibacterial components of C. asiatica inhibited the growth of S. aureus and E.coli. However, only the pure extracts of each solvent showed a wider clear zone of inhibition with S. aureus. The result further showed that distilled water was the most effective solvent in extracting the antibacterial component of C. asiatica when tested against E.coli. Statistically, the results showed that there was no significant difference in the habitat both for the phytochemical content and antibacterial property.

Keywords: gotu kola (centella asiatica), phytochemical content, zone of growth inhibition

Introduction

The Philippines abounds with plants that have been known to have medicinal properties and have been used for their curative powers throughout the ages (Asis, 1996). Recently, much interest in the field of medicinal plants has started throughout the world, and many countries have already come to realize not only their potential as means of alleviating health problems, but also their economic value.

Renewed and accelerated interest in herbal medicine in the Philippines today is of special interest because of the rich heritage of these plants. It is observed that these plants are useful among a large portion of our people, mainly in the rural areas where lack of medicines is felt; however, there is lack of available information on these (Ladion, 1985). Efforts made to alleviate our public health problems are gigantic but not nearly enough to meet the needs of a rapidly growing population.

The ability of the plant to transport both organic and inorganic nutrients, including water, throughout the plant body is critical in determining the ultimate structure and function of its component parts, as well as the development and form of the plant as a whole (Raven, et. al., 1986).

Land plants face a variety of problems. In most regions, they are subjected to periodic drought and to rapid diurnal and seasonal changes in temperature. They must survive during seasons unfavorable to their growth, they must often grow on substrates of unfavorable mineral composition, and they are subjected to the action of gravity which affects land plants much more strongly than it does water plants (Raven, et al., 1986).

Thus, plants need the right ecological conditions so that they can thrive. Often, however, the plants have to make the best of what their environment offers them (Mühlberg, 1982).

Centella, a specific variety of Gotu Kola (Centella Asiatica) is a slender, creeping plant that grows only in Madagascar while other varieties are found in India and neighboring countries. Depending on the environment, the form and shape of Gotu Kola can change dramatically. In shallow waters, it will form floating leaves; in dry locations the leaves are small and thin with numerous roots (http://www.nutriniart.com, July, 2000). Locating sources for high quality Gotu Kola requires constant effort. Due to its nature of growing in marshy areas, it is common to harvest some dirt, unintentionally, along with the leaves and roots of the plant. Much of it grows in unsanitary conditions. In some locations raw sewage runs in exposed ditches, potentially contaminating stands of Gotu Kola growing in low lying areas. Samples coming from this source were found to contain high content of coliform (originating from excrement), mold and yeast (www.drugstore.com). Cleaner sources of Gotu Kola must be considered.

Gotu Kola, Centella Asiatica, has been widely used for a number of conditions, especially in traditional eastern health care (http://www.nutrimart.com/gotukola.htm). Researches made on this plant in a foreign country revealed that it had significant results in the healing of skin, other connective tissues, lymph tissue, blood vessels, and mucous membranes. It was found that it contains several glycosides that exhibit wound healing and anti-inflammatory activities (http www.gotu.htm). However, there has been no report or any scientific study about the plant constituents and antibacterial property particularly those taken from different habitats. While most studies on Gotu Kola were done abroad, no study has yet been done with our local variety. Hence, the researcher is motivated to investigate the phytochemical content of Gotu Kola grown in dry and watery area abundant in Zamboanga City, as well as their antibacterial property.

Review of Related Literature

Plants are a rich source of chemicals that are beneficial to man in a variety of ways. Besides contributing vitamins, minerals, and fiber, these plant foods also contain phytochemicals, as scientists and doctors call them or naturally occurring plant chemicals - from the Greek "Phyto" meaning "plant" (E. Excell, 1996) that promote wellness and decrease the risk of many diseases. An array of ever expanding of previously unknown plant compounds with hard to pronounce names are being uncovered. Names ranging from lignins inflax to phenolics in wine and grapes and saponins in oats and alfalfa, anthocyanins, lycopenes, xanthenes, isothiocyanates, and sulphoraphane.

As early as 1800, chemists and plant biologists had analyzed plants and demonstrated that certain chemical elements were absorbed from the environment. Opinions differed, however, on whether the absorbed elements were impurities or constituents required for essential functions (Raven, et. al., 1986). Because inorganic nutrients fill such basic needs and are involved in such fundamental processes, the deficiencies affect a wide variety of structures and functions in the plant body.

According to Asis, 1971, within a given dryland habitat plants often vary in growth habit. For example, in an abandoned patch, herbs, vines, and shrubs may grow side by side. Because of this type of environment, which is entirely different from that of water, the plants have varied reactions upon themselves and upon the various factors surrounding them. Plants must obtain from the environment the specific raw materials required in the complex biochemical reactions necessary for the maintenance of their cells and for growth. Plant nutrition involves the uptake from the environment of all the raw materials required for essential biochemical processes, the distribution of these materials within the plant, and their utilization in metabolism and growth (Raven, et.al., 1986).

Metabolism is a characteristic feature of every living form. All physiological processes involve intake, transformation and expulsion of organic and inorganic substances combined with energy changes. Living organisms such as animals are dependent on existing organic combinations. Their manner of nutrition is heterotrophic. Plants, on the other hand, are autotrophic. They are able to provide themselves with food by synthesizing inorganic into organic substances (Mühlberg, 1982).

Nature is so wonderful that it provides everything we need. Many conditions and sickness can be helped by the use of plants. Many of the healing properties in the plant kingdom have yet to be discovered. Plants provide remedies that are more suitable for the human body than the chemical products and are the best agents for treating diseases. Plants have properties that are conducive to health. Plants have an advantage over chemical and inorganic medicines. Plants do not leave undesirable effects (Gerolaga, 1995).

Dayrit et al (1987) investigated the phytochemical constituents of the leaves of vitex Legundo L. commonly called Lagundi. From the leaves, four flavonoids (casticin, chrysophenol D, luteolin and isoorientin which are common plant acid) and a sugar (D- fructose) were extracted and isolated.

Primitive tribes in various parts of the world have known that physiological effects can be obtained by eating or chewing the leaves, roots, or bark of certain plants. The effects vary with the plants. Some, such as opium, are addictive drugs. Still others are deadly poisons, such as the leaves of the belladonna plant.

Alkaloids are particularly abundant in higher plants, insects, amphibians, and fungi and much less common in mammals. Typical examples include cocaine, nicotine, mescaline and strychnine. Most alkaloids have biological activity of some kind, and many have been exploited for their pharmacological properties. (Mann J., 1994).

Alkaloids are mostly compounds of high molecular weight, usually solid, and crystallize readily, although some of them are liquids. They are usually poorly soluble in water. Alkaloids are present in various organs (roots, leaves, stems, flowers, fruits) and tissues of plants. (Doby Geza, 1965).

Alkaloids, like most other substances having a nitrogen base are precipitated by the salts of the heavy metals (Johanssen, Donald Alexander, 1940). Leucoanthocyanins is under the flavonoid groups. Colorless compounds that occur in the same cells in which the anthocyanins are present. Both water-soluble and water-insoluble leucoanthocyanins may be distinguished. (Doby Geza, 1965). Flavonoids represent a very widespread group of water-soluble phenolic derivatives, many of which are brightly colored being red, crimson, purple and yellow. Leucoanthocyanins are sometimes called proanthocyanins because they are easily converted into anthocyanins when heated in the presence of acid.

The anthocyanins represent major contributors to the colors of many edible fruit. Variation in color of different varieties of fruit, example "black" and "white" cherries, is usually due to quantitative differences, rather than changes in the nature of the pigments present. Tannins are a group of compounds present in some plants, which can tan animal skins to produce leather. The word tan is derived from the Latin from of a Celtic word for oak, an extract of oak bark being a common tanning agent. Tannins are derived into two main groups: hydrolysable tannins and condensed tannins (non-hydrolysable). Hydrolysable tannins contain a core of a polyhydric alcohol, usually, if not always glucose, which is esterified with either gallic acid to form the gallotannins or with hexahydroxydiphenic acid to form the ollagitannins. Condensed tannins, however, are made up of only phenols of the flavone type and are often called flavolans because they are polymers of flavans. They never contain sugar residues compared to hydrolysable tannins. (Goodwin et al., 1983).

At present, many researchers are conducting experiments on medicinal plants that have not been tested yet. Today, many species of plants are being hybridized hoping to find strong derivatives that are capable of destroying the rapidly evolving new strains of pathogen. Antiseptics are agents that destroy or inhibit microorganisms when applied to living tissue. It is used for the destruction of disease-causing and other organisms on the surface of the human body or in body cavities accessible from the outside. (Collier's Encyclopedia)

In a study conducted by Bojo et al (1994) extracts of Peperomia pellucida, also known as pansit-pansitan, demonstrated antibacterial activity. The results showed significant antibacterial action against Gram negative (Escherechia coli, Pseudomonas aeruginosa) and Gram-positive bacteria (Staphylococcus aureus, Bacillus subtilis). The study showed that Gram negative is strongly sensitive to the extracts.

An invitro inhibitory effect of Bixa orellana ("achuete") seed extract against S. aureus and Streptococcus pyogens was studied by Rubith Laguna in 1996. Four concentration (25%, 50%, 75% and 100%) in 80% alcohol were evaluated. The zones of inhibition were compared with the zones of inhibition produced by commonly used drugs, cloxacillin for S. aureus and Penicillin for S. pyrogens. The B. orellana seed extract did not produce a significant inhibitory effect on the test organisms as shown by the analysis of variance (ANOVA). Moreover, as the seed extract concentration increased, the zones of inhibitions for both organisms decreased.

In medical practice, microbial cultures are isolated from diseased patients to confirm diagnoses and to aid in decisions on therapy. Determination of the sensitivity of microbial isolates to antimicrobial agents is one of the most important tasks of the clinical microbiologist (Madigan, et. al., 1997).

Food and Drug Administration (FDA) regulations now control the procedures used for sensitivity testing in the United States, and similar regulations exist in other countries. A recommended agar diffusion procedure is called the Kirby-Bauer method, named after er the

workers who developed it (Madigan, et al., 1997). The disk-diffusion technique is commonly used to test the overall effectiveness of antiseptics, disinfectants and chemotherapeutic agents. This is technically straightforward and is the simplest procedure to use because it is relatively cheaper and can determine the overall effectiveness of a given agent against a given organism.

Centella is a specific variety of Gotu Kola (Centella Asiatica Hydrocotyle Asiatica) and no other varieties possess such high amounts of asiaticosides and other triterpenes, the term "centella" is reserved for just this variety; "gotu kola" is used for all other varieties. Gotu Kola is related to carrot, parsley, dill and fennel, but does not have their feathery leaves or umbrellas of tiny flowers. Instead, its creeping stem grows in marshy areas and produces fan shaped leaves about the size of an old British penny, hence, its common names Indian pennywort, marsh penny and water pennywort.

Gotu Kola is a creeping weed about 3 to 15 cm long. The stems are in the form of stolons and rhizomes. The leaf has a long, erect petiole, 3 to 20 cm long, usually flattened and sheathing at its base. The leafblade, 2 to 5 cm in diameter is palmately veined, bladder-shaped (reniform), cordate at the base, and rounded at the tip with scalloped margin. The inflorescence is a simple umbel, its peduncle in pairs or in three's, and each umbel with 2 small bracts embracing the sessile, small, bisexual and regular flowers that vary in number from 2 to 5. The flower has an inferior ovary with 5-free, stout stamens alternating with the 5 overlapping, purplish and ovate petals. The fruit (schizocarp) is small and dry with longitudinal ridge roughly reticule and containing pendulous seeds. The plant flowers from October to May (Asis, et.al., 1971).

This herb was first used in India, where it is part of Ayuverda, the traditional herbal medicine and known as Brahmi - that which aids knowledge of Supreme Reality. It is regarded as one of the most rejuvinative herbs in Ayuverda. The leaves of this swamp plant have been used to treat leprosy, cancer, skin disorders, arthritis, and tuberculosis. It has also been effectively used in the treatment of second and third degree burns. It has been shown to decrease healing time and reduce scar tissue formation.

The gotu kola herb has an important role in gynecology. It has been used successfully to promote healing after episiotomy, a surgical incision of the vulva performed to prevent tearing during childbirth. In fact, in one study reported in a French medical journal in 1996, women treated with gotu kola after childbirth healed more rapidly than those given standard treatments. In modern health care it has been used for venous insufficiency, localized inflammation and infection, and post-surgery recovery.

According to pharmacological studies, one outcome of gotu kola's complex actions is a balanced effect on cells and tissues participating in the process of healing. particularly connective tissues. One of its constituents, asiaticoside, works to stimulate skin repair and strengthen skin, hair, nails, and connective tissue (http://www.gotu.htm).

Other studies have indicated that isolated constituents of gotu kola were applied locally on wounds in laboratory rats. This resulted in healthy new connective skin tissue and increased the tensile strength of the flesh, as well as decreased the size of the wound area. Asiaticoside, a constituent of gotu kola was injected intra-muscularly or implanted directly into mice, rats, guinea pigs, and rabbits. It produced a rapid thickening of the skin, an increased production of white blood cells, increased growth of new blood vessels of the connective tissue and an increased growth of hair and nails (file:///c MyDocument/kkk.htm).

According to Shipard, 1990, Gotu Kola can be eaten straight from the plant, or added to salads, or chopped as a last minute garnish on a meal, like parsley. It has a light bitter flavor. But if it is chopped finely and added to salads or mashed potato, even the youngest child will not object, or the leaves can be used fresh or dried for tea, and sweetened with honey.

Research Objectives

1. Analyze the phytochemical content and zone of growth inhibition of gotu kola (centella asiatica).

Methodology

A. Collection of Plant Sample

Fresh, young and mature leaves of Gotu Kola were handpicked for both the phytochemical and antibacterial screening. Leaves were collected in the morning when they are still fresh and its cells are turgid. The healthy looking leaves with no insect bites, abnormal signs of injury or discoloration were chosen.

The leaves were collected from two different areas, watery and dry area, at Johnston Compound, Canelar Moret, Zamboanga City. The plants leaves were washed with running water and placed in separate clean plastic bags and were brought to the Laboratory for the analysis. The screening procedure was replicated five times per analysis.

B. Preparation of Plant Extract

1. Alcohol Extraction for Phytochemical Analysis The plant leaves were cut finely and placed in clean containers. Using a digital pan balance, 500 grams of the plant material was weighed. In a graduated cylinder, 750 ml. of 80% ethyl alcohol was measured and transferred to a 1,000 mL. Erlenmeyer flask. The finely cut plant material was osterized with a little amount of the 80% ethyl alcohol which was added slowly until all the 750-ml have been used. This was allowed to stand for 48 hours in the 80% ethyl alcohol in a beaker and was covered with a foil.

The mixture was poured while stirring through a Buchner funnel lined with filter paper, fitted to a suction flask. The pulp was manually expressed to remove the excess solvent. The excess solvent was included in the filtration process. Fresh portions of 80% ethyl alcohol was added to rinse the flask and plant material. The rinsings were added to the extract. The plant residue was discarded.

The plant extract was concentrated to about 50 ml. in a rotary evaporator. The equivalent of plant material per ml. of the extract was calculated as follows:

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

2. Aqueous Extraction for Microbial Analysis About 200 grams of plant leaves was weighed then cut into small pieces. These were osterized in a blender with a little amount of sterile distilled water from a measured volume of 550 mL. The slurry was washed and transferred into a 1000-mL beaker. The beaker was covered with foil and the plant extract was allowed to stand with occasional stirring for 24 hours. The extract was filtered into a 500

ml. Erlenmeyer flask. Three different concentrations were prepared from the aqueous plant extract: 75%, 50%, and 25% respectively. These were covered and refrigerated until ready for use.

3. Alcohol Extraction for Microbial Analysis About 300 grams of the plant leaves were cut into small pieces and were ground further using a sterile blender. The ground leaves were placed in an Erlenmeyer flask and sufficient amount of 95% ethyl alcoholwas added to completely submerge the leaves. It was covered and soaked for 48 hours. Using a Buchner funnel, the leaves were filtered. The flask with the leaves was rinsed with fresh portions of alcohol. The washings were combined with the filtrate. The plant residue was discarded. The filtrate was concentrated to about 50 mL in a rotary evaporator. The filtrate was divided into four portions. The first portion was diluted to a concentration of 75%, the second portion, 50% the third portions, 25%. The remaining portion was used as pure extract. The same procedure for extraction was repeated using 70% ethyl alcohol.

C. Phytochemical Screening

1. Alkaloids About 2.0mL of plant extract was evaporated to a syrup consistency in an evaporating dish over a water bath. To the concentrated extract, 5 mL of 2 M Hydrochloric acid (HCI) was added while stirring constantly for five minutes. This was cooled to room temperature.

About 0.5 gram of powdered sodium chloride (NaCl) was added to the concentrated extract. This was stirred and filtered. Enough freshly prepared 2 M HCI was added to wash the filter paper until the filtrate reach a final volume of 5 mL.

To 3.0 ml. of the filtrate, enough 28% ammonia (NH;) was added dropwise to render the solution alkaline to litmus paper. The alkaline solution was extracted three times with 10-ml. portions of chloroform (CHCI,) per extraction. The combined chloroform extracts were evaporated in an evaporating dish over a water bath.

The residue was dissolved in 5 mL of 2 M HCI, stirring over a steam bath for two minutes, and cooled to room temperature. The solution was filtered and the filtrate was divided into two equal portions. To one portion, a few drops of Mayer's reagent were added. Any turbidity that formed was observed and recorded.

The result was recorded as follows:

+ = for slight turbidity

++ = for definite turbidity

+++ = for heavy precipitate

The same test was done to the second portion, except that this time, instead of using Mayer's reagent, a few drops of Wagner's reagent were also added. The same procedure was used to record the result, (+), (++) or (+++). In both tests the absence of precipitate or turbidity was assumed as absence of alkaloids in the plant material. A (+), (++), or (+++) recorded for the above tests was a positive result for the presence of alkaloids.

Results and Discussion

Phytochemical Screening

Eighty percent ethyl alcohol was used to extract the phytochemical components of Centella Asiatica. Table 1 shows that the tests for alkaloids gave a negative result using Mayer's reagent, but positive (+) for Wagner's reagent in all trials, both in wet and dry areas. This could mean that alkaloids present in C. asiatica may not be enough as the extract gave negative results in all trials with Mayer's reagent. It can be assumed that the alkaloid present in C. asiatica is more attracted to the lighter metal K present in Wagner's reagent than the heavy metal Hg that is present in Mayer's reagent. The alkaloid in C. asiatica precipitated with the K metal present in Wagner's reagent instead of Hg, which is present in Mayer's reagent.

Saponins are glycosides that are characterized by their ability to froth when the aqueous solution is agitated. A honeycomb froth that formed above the surface of the liquid and had persisted after thirty minutes indicated the presence of saponins. C. asiatica taken from both wet and dry areas gave a positive (+) result for saponins.

Treatment of a defatted alcoholic plant extract with concentrated HCI and subsequent reduction with Mg metal that gave colors ranging from orange to red to crimson and magenta occasionally to green or blue indicated the presence of flavonoids.

C. asiatica tested positive (+) on flavonoids giving a brownish upper layer and a light green lower layer.

The test for the presence of leucoanthocyanins yielded positive (+) results. All tubes exhibited a strong red coloration indicating the abundance of leucoanthocyanins,

The C. asiatica extracts gave a positive result with the Gelatin Salt reagent showing formation of a slight turbidity and were recorded "+". This result further showed the absence of polyphenolic compounds. The FeCl, test confirmed the presence of hydrolysable tannin However, the presence of condensed tannin was negative since a brownish-green color that is indicative of its presence was not observed.

The test for the presence of anthraquinones yielded negative results both in the Bontrager and Modified Borntrager's test. All tubes showed no reaction with these tests

Table 1. The Results of the Phytochemical Screening of the Alcoholic Extract of C. Asiatica.

Plant Constituents		Alkaloids		Saponin	Flavonoids	Leucoanthocyanins	Tannins & Polyphenolic Copals			Anthraquinones	
Extract	Run	Mayer's	Wagner's				Cielatin	$FeCl_3$	Polyphenolle	Bomtrager's	Modified B.
Wet	1	-	+	+	+	+	+	+	-	-	-
	2	-	+	+	+	+	+	+	-	-	-
	3	-	+	+	+	+	+	+	-	-	-
	4	-	+	+	+	+	+	+	-	-	-
	5	-	+	+	+	+	+	+	-	-	-
Dry	1	-	+	+	+	+	+	+	-	-	-
	2	-	+	+	+	+	+	+	-	-	-
	3	-	+	+	+	+	+	+	-	-	-
	4	-	+	+	+	+	+	+	-	-	-

	5	-	+	+	+	+	+	+	-	-	-

Microbiological Assay

The solvents used to extract the antibacterial component of C. asiatica were distilled water, 90% ethyl alcohol and 70% ethyl alcohol. Three concentrations were prepared from each solvent extract: 75%, 50% and 25%. The disc diffusion technique was used to determine whether the plant extract possesses antibacterial property.

Table 2. Average Measurement of the Zone of Growth Inhibition (in mm)

Plant Extracts	Dry Area						Wet Area					
	Test Organism						Test Organism					
	S. aureus			E. coli			S. aureus			E. coli		
1. Aqueous Extract	Control	Extracts	Difference	Control	Extracts	Difference	Control	Extracts	Difference	Control	Extracts	Difference
Pure extract 75%	0	6.7	6.7	2.0	3.0	1.0	0	6.8	6.8	1.9	2.9	1.0
extract 50% extract	0	4.3	4.3	2.0	4.0	2.0	0	4.4	4.4	2.0	4.2	2.2
25% extract	0	2.3	2.3	2.0	5.6	3.6	0	2.9	2.9	2.0	5.5	3.2
	0	1.4	1.4	2.0	7.0	5.0	0	1.4	1.4	2.1	7.1	5.0
2. 95% alcohol Extract												
Pure extract 75% extract 50% extract												
25% extract	4.0	7.0	3.0	9.0	7.5	1.5	4.1	7.0	2.9	9.0	7.5	1.5
	4.1	6.5	2.4	9.0	7.7	1.3	4.1	6.5	2.4	8.9	7.7	1.2
	3.9	5.1	1.2	9.0	8.1	0.9	4.0	5.0	1.0	8.9	8.0	0.9
3. 70% alcohol Extract	4.0	4.8	0.8	9.0	8.4	1.6	4.0	4.9	0.9	9.0	8.4	0.6
Pure extract 75% extract 50% extract												
25% extract	3.0	6.5	3.5	9.4	8.9	0.5	3.1	6.6	3.5	9.5	8.8	0.7
	2.9	5.7	2.8	9.4	9.0	0.4	3.0	5.6	2.6	9.4	8.9	0.5
	3.0	5.4	2.4	9.4	9.1	0.3	3.0	5.4	2.4	9.5	9.1	0.4
	3.1	4.5	1.4	9.5	9.2	0.3	3.0	4.6	1.6	9.5	9.2	0.3

Table 2 shows the average measurement of the diameter of the clear zone of the two test organisms using the aqueous, 95% ethyl alcohol and 70% ethyl alcohol plant extracts both in wet and dry areas. The result shows that the C. asiatica extracts inhibited the growth of S. aureus and E. coli. This was indicated by the presence of the clear zone of inhibition. The clear zone of inhibition indicates that C. asiatica extracts contain substances that can inhibit the growth of the said test organisms. As stated by D.C. Benjamin (1991), in his book Essentials of Medical Microbiology, the larger the size of the zone of inhibition of bacterial growth, the more sensitive the organism is to the drug. The different diameter of the clear zone as shown in the table indicates the varying inhibiting property of the solvents used as well as the susceptibility of the organism.

The result shows that as the dilution of the plant extracts decreases in all solvents used using S. aureus as test organism both in dry and wet areas, the diameter of the zone of inhibition decreases. It further shows that all extracts have a wider zone of inhibition. ranging from 1.4-7.0 mm as compared to the control with a range of 0-4.1 mm. Furthermore, the control in the 95% ethyl alcohol solvent used has wider zones of inhibition, followed by the 70% ethyl alcohol with an average of 4.0 mm and 3.0 mm, respectively.

Using E. coli as test organism, the result shows that as the dilution decreases in all solvents used both in dry and wet areas, the diameter of the zone of inhibition increases, meaning, a wider zone of inhibition. It further shows that the aqueous extracts have a wider zone of inhibition with an average value of 4.89 mm as compared to the control with only 2.0 mm both in dry and wet areas. However, for the 95% and 70% ethyl alcohol extracts the zone of inhibition of the control is wider as compared to the extracts. The result also further revealed that in the control, the diameter of the zone of inhibition is widest in the 70% ethyl alcohol with an average value of 9.425 mm for dry area and 9.475 mm for wet area. The 95% ethyl alcohol ranks second with an average measurement of 9.0 mm for dry area and 8.95 mm for the wet area, and the least is the aqueous solvent having an average diameter of 2.0 mm both in dry and wet areas.

Table 3. Summary of Two Way ANOVA Comparing the Zone of Inhibitions of S. aureus on aqueous, 95% ethyl alcohol and 70% ethyl alcohol with different dilutions on two areas.

Source of Variations	Sum of Squares	df	Mean Square	F-ratio	F-crit 0.01	Interpretation
Aqueous Extracts	95.09	3	31.70	150.95	5.29	Significant
Area	0.22	1	0.22	1.05	8.53	Not
Interaction	0.29	3	0.10	0.48	5.29	Significant
Error	3.29	16	0.21			Not
Total	98.89	23				Significant
95% et. Alc. Extracts	20.02	3	6.67	3705.56	5.29	Significant
Area	0	1	0	0	8.53	Not
Interaction	0.01	3	0.0033	1.83	5.29	Significant
Error	0.03	16	0.0018			Not
Total	20.06	23				Significant

70% et. Alc. Extracts	12.390	3	4.13	16.52	5.29	Significant
Area	0.03	1	0	0	8.53	Not
Interaction	0.04	3	0.01	4	5.29	Significant
Error	12.46	16	0.0025			Not
Total		23				Significant

Table 3 present the results of the comparison of the zone of inhibition of S. aureus

in aqueous, 95% ethyl alcohol and 70% ethyl alcohol plant extracts with different dilutions in two areas using Two-Way ANOVA.

The result shows that there is no significant difference in the antibacterial property of C. asiatica grown in dry and watery areas in all of the plant extracts using different solvents with different dilutions. This implies that regardless whether C. asiatica was taken from dry or wet area, it has the same antibacterial property.

However, the result revealed that there was a significant difference in the antibacterial property of C. asiatica extracts diluted to different concentrations. This is indicated by the low value of F-crit, which is 5.29 at 0.01 level of significance as compared to the values of the F-ratio for the aqueous extracts, 95% ethyl alcohol extracts and 70% ethyl alcohol extracts which is 150.95, 3705.56 and 16.52, respectively

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