Print ISSN 2094-1064
Online ISSN 2244-0437

Liceo Journal of Higher Education Research
doi: http://dx.doi.org/10.7828/ljher.v13i2.1063

Chromolaena Odorata Leaf Extract Hand Sanitizer

MERCEDITA S. AQUINO
ORCID NO. 0000-0003-0435-4298
msaquino@liceo.edu.ph

Liceo de Cagayan University
Cagayan de Oro City, Philippines

ABSTRACT

Hygiene is imperative to maintaining health and preventing disease. Hand washing with soap and water helps in the prevention of transmission of diseases, a measure to prevent nosocomial infection in the healthcare setting where opportunistic bacterial like *Staphylococcus aureus* and *Escherichia coli* are acquired. This study aimed to formulate liquid solution containing the leaf extract obtained from *Chromolaena odorata* (L.) King & Robinson family Asteraceae, that has potential antibacterial property. The antibacterial property of the formulation was tested employing the disc diffusion method against *Staphylococcus aureus* (gram positive) and *Escherichia coli* (gram negative). The diameters of the zones of inhibition were measured. The tests of the different concentrations of the formulated C. odorata hand sanitizer solution were carried in one run. The three different concentrations were 1.0 gram, 0.75g, and 0.5g of ethanolic extracts per 100-milliliter preparation. The positive control used was ampicillin, and the negative control was isopropyl alcohol. The tests revealed that there were no zones of inhibition observed against the test organisms on the three different concentrations. The formulated *Chromolaena odorata* leaf extract hand sanitizer liquid solution containing 1.0 g, 0.75g, and 0.5g of ethanolic extracts has no antibacterial property against *Staphylococcus aureus* and *Escherichia Coli*.

Keywords: hand sanitizer, *Chromolaena odorata*, antibacterial property

INTRODUCTION

Bacteria are found everywhere. There are millions that are found and living on the skin, telephones, doorknobs, air and more They spread through the hands and gain access to the body through broken skin and cause illness. One of the best ways to prevent the spread and acquiring illness is to practice hand hygiene.

Hand hygiene is a condition and practice applied to the hands to maintain good health and prevent diseases which are attained by applying hand antiseptic or surgical wash. It is believed to be an excellent and economical way to promote and preserve health (Babebek et al. 2014). Keeping the hands clean prevent transmission of nosocomial infections in the hospitals and healthcare settings (Ochato et al. 2015), likewise in non-clinical settings specifically in the spread and transmission of cold and flu viruses (White 20015).

Nosocomial infections are acquired in the hospital, in nursing homes and other health care facilities. These infections may result from microorganisms in the hospital, weakened host and chain of transmission from patient to patient. Although every effort is made to kill or check the growth of microorganisms in the hospital, the hospital environment is the main reservoir for a variety of pathogens. Most of the microbes that cause nosocomial infections do not cause disease in healthy people but are pathogenic only for individuals whose defenses have been weakened by illness or therapy. They gain access to broken skin or mucous membrane and cause opportunistic infections.

Staphylococcus aureus and *Escherichia coli* are among the microorganisms involved in most nosocomial infections (Tortora et al. 2002). Infectious sites are a urinary tract, surgical wounds, respiratory tract, skin, blood, gastrointestinal tract, and central nervous system (Wani 2013). *S. aureus* grows comparatively well under conditions of high osmotic pressure and low moisture. They can grow and survive in nasal secretions (many of us carry the bacteria in our noses), on the skin. They also thrive in some foods which high osmotic pressure (such as ham and other cured meats) and in low-moisture foods that tend to inhibit the growth of other organisms. It is one of the most exquisitely tuned microorganisms for causing disease in humans. It is responsible for a long list of different diseases. It can cause pneumonia, food poisoning, serious bloodstream infections, bone infections, toxic shock syndrome and meningitis (Cowan 2015). *E. coli* an opportunistic pathogen ordinarily does not cause disease in their usual habitat in a healthy person but may do so in a different environment. It is not usually pathogenic; however it can be a cause of urinary tract infections, and certain

strains produce enterotoxins that can cause traveler's diarrhea and occasionally cause serious foodborne disease (Tortora 2002). Dozens of different strains of *E. coli* exist, many of which cause no disease at all. *E. coli* 0157: H7 and its close relatives are the most virulent of them all. This *E. coli* strain which is referred to as Shiga-toxin producing *E. coli* (STEC) is transmitted by ingestion of contaminated and uncooked beef. The *E. coli* species that cause urinary tract infection (UTIs) are those that exist as normal biota in the gastrointestinal tract. These uropathogenic *E. coli* (UPEC) are called Extraintestinal. Pathogenic *E. coli* (ExPEC) secure themselves in the gastrointestinal tract using specific adhesins on the ends of the fimbriae. They can reside here without causing disease but also travel to the locations in the body where they can cause disease (Cowan 2015).

The normal flora and transient flora identified as bacteria establish themselves inside our bodies, on the surface and hands. The former may establish more or less permanent residence, but they do us no harm. The transient flora may be present for several days, weeks, or months and then they disappear. These are the kind of bacteria that are often acquired by Health Care Workers (HCWs) however they and can be removed by handwashing. The transient flora is transmitted to the patients whom the HCWs are in contact with, or through the contaminated surroundings close to the patient. Resident flora, which thrives to deeper layers of the skin, are difficult to remove (Boyce 2002).

The most important means of preventing transmission of infectious agents from person to person or from region of high microbial load such as the mouth, nose or gut to potential sites of infection is washing of hands (Beale 2011). The concept of cleansing hands with an antiseptic agent probably emerged in the early 19th century. One breakthrough in surgical technique in the 1800's was the finding that the incidence of postsurgical infections decreased if surgeons washed their hands before operating. A study by a French pharmacist in 1822 revealed that solutions containing chlorides of lime or soda could be used to deodorize dead human body. The pharmacists further proved that the solution could be used as disinfectant and antiseptic. Relative to the discovery, a paper published in 1925 stated that the liquid chloride solution could prevent contagious diseases by applying on the hands (Boyce 2002).

Hand sanitizer is useful in the reduction of bacteria when one is unable to wash with soap and water. Most claims to kill 99.9% bacteria of common harmful germs (Boyce 2002) encountered in the day-to-day activities. Nowadays, hand sanitizer formulations are available, made handy carried and applied by individuals anywhere and anytime when needed. Its use is highly acceptable by

travelers and is associated with a reduction in the incidence of travelers' diarrhea and vomiting (Henriey et al. 2014). It is the aim of this study to formulate hand sanitizer solution with potential antibacterial property obtained from the plant, *Chromolaena odorata* instead of or in conjunction with traditional hand-washing (with plain soap and water).

According to Tyler (1988), plants contain secondary metabolites such as glycosides, phenols, tannins, alkaloids and provide most of the pharmacologically active products (Shah and Seth 2010). The ethanolic extract of *Chromolaena odorata* contains tannins, flavonoids, saponins which showed antimicrobial activity (Vital et al. 2009), antifungal activity (Ngane et al. 2006), anti-inflammatory, analgesic and antipyretic activities (Owoyele 2008). Its essential oil (Owolahi et al. 2010) with chief components: α pinene and β pinene and which showed antibacterial property against *Bacillus cereus*. Apparently, plant extracts containing tannins and flavonoids exhibit antimicrobial property (Rajalakshmi 2016; Owolahi et al. 2010). Studies on flavonoid contents in plants showed antioxidant activity, by inhibiting the di (4-tretocylphenyl-1-picryl-hydrazyl, DPPH) scavenging activity (Abdalaziz et al. 2016; Vijavaraghavan et al. 2013; Srinivasa 2009). Interestingly, extract from *C. odorata* contains a mixture of powerful antioxidant compounds that may be one of potential mechanism contributing to enhanced wound healing (Toan-Thang PHAN 2001). Based on studies, the antimicrobial property of the *C. odorata* leaves extract combined with suitable excipients can be prepared as hand sanitizer.

FRAMEWORK

Plants have been the origin of many drugs. They contain secondary metabolites that provide most pharmacologically active products (Tyler 1988) which can be extracted and isolated using suitable solvent and subsequently formulated into a drug product. *Chromolaena odorata* (L.) King and Robinson (Asteraceae) leave contain tannins and flavonoids that have antimicrobial property (Vital et al. 2009). A hand sanitizer liquid solution containing the methanolic extract obtained from Chromolaena odorata was formulated into three (3) concentrations as namely 1 gram, 0.75 and 0.5g in 100 milliliters. The antibacterial property of the formulated hand sanitizer solutions against *Staphylococcus aureus* and *Escherichia coli* was determined.

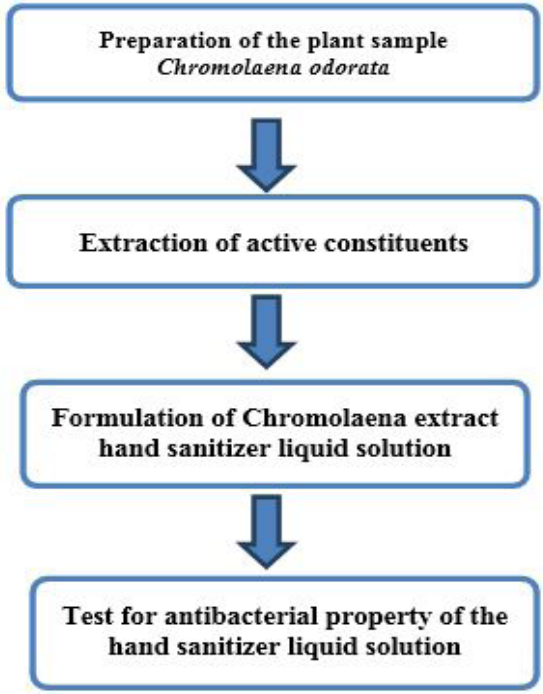

Figure 1. Research Flow Chart

OBJECTIVES OF THE STUDY

Several studies claimed that extract obtained from *Chromolaena odorata* contains tannins and flavonoids that are responsible for its antibacterial property. This study aimed to formulate hand sanitizer liquid solution that contains leaf extract of *Chromolaena odorata* and determine its antibacterial property against *Staphylococcus aureus*, gram-positive bacteria and *Escherichia coli*, gram-negative bacteria.

MATERIALS AND METHODS

Collection and Identification of the Plant

Leaves of *Chromomalaena odorata* (L.) R. M. Family: Asteraceae were collected from Zone 2 Barra, Opol, Misamis Oriental. *C. odorata* was identified by Hannah P. Lumista, Curator, Botany Division and confirmed by Florfe M. Acma, Botanist/Head, University Museum, Central Mindanao University, University Town, Musuan, Bukidnon

Chromolaena odorata (L.)(formerly *Eupatorium odoratum* L.), is a perennial plant of the family Asteraceae (Compositae). It is a scrambling, flowering shrub locally known as hagonoy. It grows to 3—7 m in height bush when growing in the open farmland. It is a rapidly growing and strongly scenting perennial shrub. The weed goes by many common names including Siam weed, Devil weed, French weed and known as 'AkintolaTaku' by the Yorubas of Nigeria. The plant is native to North America and Central America. It was introduced into the tropical regions of Asia, Africa, and the Pacific, where it is invasive (Lawal 2015). The plant easily widens its range due to its impressive short- and long-distance distribution. A vertical stand is formed mostly in disturbed areas, grasslands, idle, uncultivated plowed land areas, and forestry plantations. *Chromolaena odorata* is competitive and considered as environmental and the world's worst weeds.

Taxonomy of the plant: (CABI Invasive Species Compendium)

Domain: Eukaryota
Kingdom: Plantae
Phylum: Spermatophyta
Subphylum: Angiospermae
Class: Dicotyledonae
Order: Asterales
Family: Asteraceae
Genus: Chromolaena
Species: Chromolaena odorata

Preparation of Methanolic Extract

Chromolaena odorata leaves were air dried and ground to a coarse powder using a dry mill. The methanolic leaf extract was prepared by macerating 300 g of powdered leaves in methanol for 72 hours with occasional agitation. The mixture was filtered after the required time of maceration to extract the constituents

soluble in methanol. The solvent methanol was removed under vacuum using a rotary evaporator. The yield (concerning the dry powdered material) of *C. odorata* leaves extract was 6.0grams (2%).

Formulation of Chromolaena Extract hand sanitizer liquid solution

To formulate the hand sanitizer liquid solution, *C. odorata* leaves extract was added to the mixture of isopropyl alcohol and water until a clear solution was obtained with constant stirring. Glycerin was added to the mixture to enhance the solubility of the extract. Propyl paraben and lavender oil were added slowly to preserve and to give scent to the preparation, respectively. A homogeneous mixture was obtained after continuous stirring and was placed in the airtight container. The preparation of the 0.75g and 0.50 extract employed the same procedure, and the appropriate amount of the excipients were computed accordingly (Table 1).

Table 1. Composition of Hand Sanitizer

Ingredients	Quantity
C. odorata leaves extract	01.00 g
Isopropyl alcohol	63 .00 mL
Glycerin	03.00 mL
Distilled water	32.00 mL
Propyl paraben	00.5 mL
Lavender oil	00.5 mL

In vitro Antibacterial Property by Disc Diffusion Method

The antibacterial property of the formulated hand sanitizer liquid solution was determined using the disc diffusion method against *Staphylococcus aureus* (gram positive) and *Escherichia coli* (gram negative). The determination was carried out by inoculating the bacteria on different plates into MH agar at 37^0 C for 6 hours. The filter paper disc saturated with the formulated hand sanitizer was placed on the surface of each inoculated plates. The positive control used was ampicillin,

and the negative control was isopropyl alcohol. The tests were carried in one run for three concentrations of the formulated *C. odorata* leaf extract hand sanitizer containing 1 gram, 0.75g, and 0.5g of extracts. For 24 hours at 37^{0}C, the plates were incubated. After the incubation, the zone of inhibition was measured.

RESULTS AND DISCUSSION

The hand sanitizer liquid solution formulated from the methanolic extract obtained from the leaves of *Chromolaena odorata* was prepared in different concentrations 1.0 gram, 0.75 gram, and 0.50 gram, per 100 mL. The hand sanitizer was tested for its antibacterial property against *Staphylococcus aureus* (gram positive) and *Escherichia coli* (gram negative) bacteria employing the disc diffusion method.

Table 2. Result of Antibacterial Tests of Chromolaena Extract Hand Sanitizer Liquid Solution

Test organism	**Zone of inhibition (diameter mm)**				
	Concentration of the extract gram(g) / 100 milliliter preparation			Negative control	Positive control
	1g	0.75g	0.5g		
Staphyloccocus aureus (gram positive)	00 mm	00 mm	00 mm	00 mm	26 mm
Escherichia coli (gram negative)	00 mm	00 mm	00 mm	00 mm	00 mm

Table 2 depicts the result of the antibacterial test of the Chromolaena extract hand sanitizer solution in three concentrations against *Staphylococcus aureus* and *Escherichia coli*. The zones of inhibition of the three concentrations and the negative control were all zero millimeters. The ampicillin, the positive control has 26 millimeters. The zero millimeter zones of inhibition by the three concentrations and negative control imply that the Chromolaena extract hand sanitizer solution has no antibacterial property.

The concept of the formulating the leaf extract of *Chromolaena odorata* into hand sanitizer was based on the studies on its antibacterial property against *S. aureus* (Nesakumar et al. 2016 and Vital et al. 2009) and *E. coli* (Taguri et al.

2004). The isopropyl alcohol used as the solvent in an amount that does not affect the antibacterial activity of the extract. High alcohol content in hand sanitizers excessively dries the skin.

CONCLUSION

Hands are the most common mode of transmission of pathogens to patients, and proper hand hygiene can prevent healthcare-associated infections and the spread of antimicrobial resistance. Several studies claimed that the leaf extract of *Chromolaena odorata* exhibits antibacterial property. It is the objective of this study to provide a hand sanitizing formulation that contains *C. odorata* leaf extract. The antibacterial property of the *C. odorata* hand sanitizer was determined using the disc diffusion method against *S. aureus* and *E. coli* measuring the diameter of the zone of inhibition. The result of this study revealed that the formulation containing 1g. 0.75g and 0.5g of extract per 100 mL failed to exhibit zones of inhibition against the test organisms. The failure to exhibit zones of inhibition implies that the *Chromolaena* extract hand sanitizer liquid prepared in three different concentrations have no antibacterial property.

RECOMMENDATIONS

The following are at this moment recommended based on findings:

1. Study further the antibacterial activity containing a higher amount of extract in the hand sanitizer formulation; and

2. Conduct a study on the colony count of the Chromolaena Hand Sanitizer containing different concentrations of the extract.

LITERATURE CITED

Babeluk R, Jutz S, Mertlitz S, Matiasek J, Klaus C. 2014. Hand Hygiene – evaluation of three disinfectant Hand Sanitizers in a community setting. PLOS One 9(11)e:111969.doc1o.1371/Journal pone 0111969

Beale JM, Block JH, Wilson C. 2011. Gisvold's textbook of organic medicinal and pharmaceuticalchemistry.12thEdition.Wolters Kluwer/Lippincott Williams a7 Wilkins.

Boyce J, Didier and Pittet MD. 2002.Guideline for hand hygiene in health-care settings recommendations of the healthcare infection control practices advisory committee and the HICPAC/SHEA/APIC/IDSA hand hygiene task force.

CABI Invasive Species Compendium. http://www.cabi.org/isc/datasheet/23248

Cowan M. 2015. Microbiology: A System Approach 4th Edition. McGraw Hill International.

Henriey D, Delmont J, Gautret P. 2014. Does the use of alcohol-based hand gel sanitizer reduce travellers' diarrhea and gastrointestinal upset? : A preliminary survey. Available online at www.sciencedirect.comScienceDirectjournal homepage: www.elsevierhealth.com/ journals/tmid

Lawal OA, Opoku AR, Ogunwande IA. 2015. Phytoconstituents and insecticidal activity of different solvent leaf extracts of Chromolaenaodorata L., against Sitophiluszea mais Coleoptera: Curculionidae. European Journal of Medicinal Plants 5(3): 237-247, 2015,Article no.EJMP.2015.023ISSN: 2231-0894 SCIENCEDOMAIN international www.sciencedomain.org

Nesakumar D, Jayakumar C, Kumar, DK, Gandhi NN. 2016. Synthesis and toxicology studies on Chromolaena odorata (Communist Green) extract. Transactions on Engineering and Sciences ISSN:2347-1964 (Online) 2347-1875 (Print)Volume 4, Issue 1.

Ngano NA, Ebelleetame R, Ndiflor F, Byiti L, Amvamzollo PH, Bouchet PH. 2006. Antifungal activity of Chromolaena odorata (L) King & Robinson (Asteraceae) of Cameroon.Microbiology, Chemotherapy 62:103-106

Ochwoto M, Muita L, Talaam K, Wanjala C, Ogeto F. 2015. Anti-bacterial efficacy of alcoholic handrubs in the Kenyan market. Antimicrobial resistance and infection control (2017) 6:17 DOI 10.1186/s13756-017-0174-3

Owoyele BV, Oguntoye SO, Kemi D. 2008. Analgesic, anti-inflammatory and antipyretic activitiesfrom flavonoid fractions of Chromolaena odorata. Journal of Medicinal Plants Research Vol.2(9), pp. 219-225, September, 2008. Available online at http://www.academicjournals.org/JMPR

Shah B, Seth AK. 2010.Textbook of pharmacognosy & phytochemistry. Elsevier.

Suksamrarn A, Chotipong A, Suavansri T, Boongird S, Timsuksai P, Vimuttipong S, Chuaynugul A. 2004. Antimycobacterial activity and cytotoxicity of flavonoids from the flowers of Chromolaena odorata. Arch Pharm Res. 2004 May;27(5):507-11.

Taguri T, Tanaka T, Isao K. 2004. Antimicrobial activity of ten different plant polyphenols against bacteria – causing Food-Borne Disease. Biological Pharma Bulletin 27 (12) 1965 – 1969,

Toan TP. 2001. Phenolic compounds of Chromolaena odorata protect cultured skin cells from oxidative damage: Implication for Cutaneous Wound Healing. December2001 Biol. Pharm. Bull. 24(12) 1373—1379 (2001)

Tortora GJ, Funke RB, Case LC. 2002.Microbiology. An Introduction. 7th Edition. 2002. Daryl Fox

Tyler V, Brady L, Robbers J. 1988. Pharmacognosy 9th edition. Lea & Febiger.

Vijayaraghavan K, Mohammed AS, Maruthi R. 2013. Studies on phytochemical screening & antioxidant activity of Chromolaena odorata & Anonasquamosal. International Journal of Innovative Research in Science, Engineering &Technology. Vol. 2, Issue 12.

Vital PG, Rivera WL. 2009. Antimicrobial activity & cytotoxicity of Chromolaena odorata King and Robinson & Uncariaperrottetii (Merr) Extracts. Journal of Medicinal Plant Research Vol. 3(70,pp.511-518 (2009)

Wani NS, Bhalerao AK, Vikram PR, Zanje R. 2013. Formulation and evaluation of herbal sanitizer International Journal of PharmTech Research CODEN (USA): IJPRIF ISSN : 0974-4304 Vol.5, No.1, pp40-43.

White C, Kolbe R, Carlson R, Lipson N. 2005. The impact of a health campaign on hand hygiene and upper respiratory illness among college students living in residence hall. Journal of American College Health, vol 53 No. 4 https://www.ncbi.nlm.nih.gov/pubmed/15663066

APPENDICES

Plate 1. *Chromolaena odorata* plant and its habitat.

Plate 2. Preparation of *Chromalaena odorata* leaves extract and formulation of Chromolaena Hand Sanitizer