EVALUATION OF THE ANTI – STAPHYLOCOCCAL ACTIVITY OF NAUCLEA ORIENTALIS LINN

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Abstract

Traditional medicine is becoming more popular nowadays. The use of herbal or medicinal plants is accepted in the health care system provided that there efficacy and safety were tested through research. This study determined the anti – *staphylococcal* activity of the mature leaves of *Nuclea orientalis Linn.* Results show that the crude alcoholic leaf extract of the mature leaves of *Nauclea orientalis Linn*. has an anti-*staphylococcal* activity. The 100 % extract's anti-*staphylococcal* activity is comparable to Erythromycin. Furthermore, the formulated ointment from the 100% extract is also comparable to the wound healing efficacy of the Erythromycin ointment in terms of duration of healing. The findings show that *Nauclea orientalis Linn.* has antibacterial and pro-repairing actions.

Keywords: Anti – *staphylococcal activity, Staphylococcus aureus, Nauclea orientalis Linn*

Introduction

The Philippines is one of those countries blessed with abundant natural resources. Plants are all over the archipelago covering the vast plains and mountains. These plants are beneficial to all the people of the country providing them source of their basic needs and other necessities. Aside from providing food, plants have a great role in drug production. Drugs are often derived from plants and animals which are very helpful as treatment for human diseases. Various plants have proven their value as sources of potential drugs. (Ballada & Domondon, 2011).

Long before the introduction of modern medicines and Western curative methods, herbal medicines had been widely used in the Philippines. The knowledge and skills on the curative application of any given herbal

medicine has been handed down from generation to generation. Lately, interests in the use of medicinal or herbal plants are becoming more and more popular in the Philippines. Research probing the effectiveness of these plants had transformed from just being part of tradition and culture to highly sophisticated laboratory or clinical tests. This validates the efficacy of these traditionally used herbal plants to specific diseases of patients. Dasmariñas, Bayalan, Villa, & Landicho, 2008)

Much progress has been made in the rediscovery of traditional medicine and its potential for strengthening primary health care programs. This is reflected in the non-governmental organizations' community-based health programs (CBHP) effort in the past, which actively employ traditional medical practitioners and promote the utilization of traditional therapies. (Tan, Querubin, & Rillorta, 1988) In support to traditional medicine, the congress passed a bill on 1997 which is the Republic Act No. 8423. This is an act creating the Philippine Institute of Traditional and Alternative Health Care (PITAHC) to accelerate the development of traditional and alternative health care in the Philippines. Part of its objective is to encourage scientific research on and develop traditional and alternative health care systems that have direct impact on public health care. (Nolledo, nd)

Nauclea orientalis Linn. plant or "Bangkal" in Filipino is one of the many species of plants located in the Philippines. It is found chiefly in secondary forests at low and medium altitudes. The leaves are applied to boils and tumors, and the decocted bark is said to be vulnerary, antidiarrhetic, and a cure for toothache. (Guerrero, 1921 as cited in Quisumbing, 1978) In the study of Aghavamma and Rama Rao (2010), the extract from *Nauclea orientalis Linn.* leaves show dose-dependent action and inhibition of spontaneous motility (paralysis) and death of earthworms. This indicates that this plant has antihilmentic properties.

Numerous studies have been carried out to extract various natural products for screening antimicrobial property but attention has not been focused intensely in studying the combinations of these products for their antimicrobial activity. (Nita et. al, 2002) Thus, this study was conceptualized.

Staphylococcus aureus is a member of the *Micrococcaceae* family. The organism appears to be gram positive cocci in cluster. It can be recognized from other *Staphylococcal species* on the basis of the gold pigmentation of the colony and positive result of coagulase. (Lowy, 1998) There has been a dramatic increase in the number of community-based *Staphylococcus aureus* infections during the past decade (Gwizdala, et al., 2011). Moreover, Strains of methicillin-resistant *Staphylococcus aureus* (MRSA), which had been largely confined to hospitals and long-term care facilities, are emerging in the community (Chambers, 2001). *Staphylococcus*

aureus is normally found in the skin. These bacteria commonly cause most skin infections. They can look like pimples or boils. They may be red, swollen and painful, and sometimes have pus or other drainage. (National Institutes of Health, nd) Once there is a break in the skin, it can penetrate and cause wound infection. The presence of infection in the wound is detrimental to wound healing. (Edwards & Harding, 2004).

The use of plant extracts and phytochemical, both with known antimicrobial properties can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency (Artizzu et. al, 1999). In connection to this, the researchers thought that *Nauclea orientalis Linn.* leaf extract could be a possible source knowledge on its antimicrobial property, efficacy and activity.

Objectives of the Study

This study sought to evaluate the anti – *staphylococcal* activity of *Nauclea orientalis Linn.*

Specifically, it:

1. Measured the susceptibility of *Staphylococcus auerus* to the following treatments in terms of zone of inhibition:
    a. 25% mature leaves extract of *Nauclea orientalis Linn.*
    b. 50% mature leaves extract of *Nauclea orientalis Linn.*
    c. 75% mature leaves extract of *Nauclea orientalis Linn.*
    d. 100% mature leaves extract of *Nauclea orientalis Linn.*
2. Determine the significant difference between and among the different treatments;
3. Determine the anti-bacterial efficacy of *Nauclea orientalis Linn.* ointment in terms of duration of healing of induced wounds infected by *Staphylococcus aureus* as compared to the control;
4. Determine the significant difference between the anti-bacterial efficacy of *Nauclea orientalis Linn.* ointment and the control;

Methodology

The researchers used the Complete Randomized Experimental Design. This was used in the conduct of laboratory tests and the susceptibility or sensitivity test, to obtain results to test the hypotheses. The study has three phases. Phase one was the microbiological tests employing standard laboratory procedures. Phase two was the formulation of the ointment. Phase three was the determination of the anti-bacterial efficacy of the formulated ointment from the most effective concentration of the crude alcoholic leaf extract of *Nauclea orientaliss Linn.* in terms of duration of healing of induced wound infected with Staphylococcus aureus.

In testing the susceptibility of the *Staphylococcus aureus* to the different treatments, fifteen discs were distributed at random to five treatments. Each treatment have three replicates with one disc assigned to each replicate. The treatment will be the following:

a. Treatment 1 – 25 % crude alcoholic leaf extract of the test plant
b. Treatment 2 – 50 % crude alcoholic leaf extract of the test plant
c. Treatment 3 – 75 % crude alcoholic leaf extract of the test plant
d. Treatment 4 – 100 % crude alcoholic leaf extract of the test plant
e. Treatment 5 – Control (Erythromycin)

In testing for the anti-bacterial efficacy of *Nauclea orientalis Linn.* leaves ointment in terms of duration of healing of induced wounds of test animals infected with *Staphylococcus aureus*, nine guinea pigs were distributed at random to three treatments. Each treatment have three replicates with one guinea pig assigned to each replicate. The treatments will be as follows:

a) Treatment 1 – Control ointment
b) Treatment 2 – Natural Healing
c) Treatment 3 – *Nauclea orientalis Linn*. leaves ointment (most effective concentration)

Phase 1: Microbiological Testing

Harvesting Matured Leaves of *Nauclea orientalis Linn*. Matured leaves of *Nauclea orientalis Linn. was* harvested at Barangay Mameltac, San Fernando City, La Union. Then, the leaves were washed with running water before drying them under the shade for 3 (three) days. The air-dried leaves were oven dried at 60 °C prior to powdering utilizing a mortar and pestle.

Preparation of Extract. To prepare 25 % crude alcoholic leaf extract, 25 grams of powdered leaves of *Nauclea orientalis Linn.* was added into a beaker with 70 % ethyl alcohol up to 100 ml calibration mark. The 50 percent crude alcoholic leaf extract was prepared by adding 50 grams of powdered *Nauclea orientalis Linn.* into a beaker and with 70 ethyl alcohol up to 100 ml calibration mark. To make the 75 % crude alcoholic leaf extract of *Nauclea orientalis Linn.*, 75 grams of powdered *Nauclea orientalis Linn.* leaves was added into the beaker with 70 ethyl alcohol up to 100 ml calibration mark. Lastly, 100 percent crude alcoholic leaf extract was prepared by transferring 100 grams of powdered *Nauclea orientalis Linn.* powder to a beaker with 70 ethyl alcohol up to 100 ml calibration mark.

The above mixtures were stirred continuously for one hour before tightly stoppered and wrapped in aluminum foil and kept in a cool, dark place for three days with occasional agitation. After 3 days, the extract was filtered and the ethyl alcohol was evaporated.

Preparation of Filter Disc. Sterilized filter discs were used on hole punch to make small circular wells on Whatman Ashless No. 42 filter paper measuring 6.0 mm in diameter. Discs were wrapped with a clean coupon bond and sterilize in the autoclave. The discs were used to immerse or soak in the different extract concentrations of *Nauclea orientalis Linn.* After soaking the discs for 24 hours, the discs were dried and sterilized in the oven for an hour at 37 °C.

Susceptibility and Sensitivity Test. The researchers collected a discharge from an infected wound following a strict standard precaution technique. Standard precautionary techniques were used in order to avoid contamination of the specimen. Gloves, sterile specimen containers, careful preparation of culture site, and the length of time between collection and actual laboratory preparation or testing can impact the results of culture growth. In addition, each organism's growth requirements, such as oxygen, moisture, temperature and nutrients, were considered. The discharge was brought to the laboratory for bacterial culture. After, the researcher isolated *Staphylococcus aureus* colony from the bacterial culture. After isolation the bacteria, Grams staining and coagulase test were perform to confirm the bacteria. Finally, the susceptibility and sensitivity test was performed using the Mueller Hinton Agar (MHA) plate. The measure of zone of inhibition determined the efficacy of the leaf extract against *S. aureus.*

Phase 2: Formulation of Ointment

The procedure of making herbal ointment adopted is from Barros (1999). The researcher used the concentration of the crude alcoholic leaf extract of *Nauclea orientalis Linn.* with the most effective bactericidal activity based on the susceptibility test in the formulation of the ointment. The ointment was formulated aseptically to avoid contamination that may affect its efficacy.

Phase 3: Antimicrobial efficacy Test of *Nauclea orientalis Linn.* Ointment

The researchers used nine male similar guinea pigs weighing approximately 350 – 400 grams. The nine guinea pigs were individually haused and contained in the individual cages for three days. The guinea pigs were brought to the veterinarian for proper physical examination prior to incision. With the assistance of a veterinarian, superficial wounds were induced using a scalpel. The superficial wound of nine guinea pigs was induced with 0.25 ml of Trypticase Soy Broth (TSB) of cultured

Staphylococcus aureus. Each guinea pig was patched with sterile gauze and a sterile tape on the wound site. The superficial wounds were incubated for three days. Right after the incubation period, the researcher removd the gauze and inspect the wounds with redness, swelling, pus formation, and infection caused by the bacteria induced to them.

The application of the ointment was then initiated. The researchers applied a thin layer dosage or a swabful (125 mg) of the ointments (the most effective concentration and the control ointment) using individual cotton buds to animal replicates of Treatments 1 and 3 while no treatments was given to replicates of Treatment 2 group. Then the wounds were covered again with sterile surgical gauze and tape. The same procedure of the application of ointments followed three times a day (6 am, 12 noon and 6 pm).

Careful observation was needed by the researchers to identify the healing of the induced wounds of the experimental animals. Duration of healing was the measure of efficacy of *Nauclea orientalis Linn.* leaves ointment. The researchers counted the number of actual days until the wound is completely healed to determine the duration of wound healing.

Treatment and Analysis of Data

The data gathered were tabulated and computed statistically using One-Way Analysis of Variance (ANOVA). Tukey's HSD Test was also used.

Results and Discussion

Susceptibility and Sensitivity Test

Table 1 shows the measurement of the zone of inhibition of *S. aureus* as affected by the different treatments. As shown, the 25% crude alcoholic leaf extract registered the following zone of inhibition of 11 mm for the three replicates. On the other hand, 50% registered a zone of inhibition of 12.6 mm for replicate 2 and 12 mm for replicates 1 and 3 with a mean of 12.2 mm. Further, 75% extract showed 15 mm, 18 mm and 11 mm for replicates 1, 2, 3 respectively with a mean zone of inhibition of 14.67 mm. The 100% exhibited 20 mm for replicates 1 and 2 and 14 mm for replicate 3 giving a mean of 18 mm. Lastly, the control (Erythromycin) showed 21 mm for replicate 1 and 22 mm for replicates 2 and 3 with a mean of 21.67 mm. This implies that the 100% crude alcoholic leaf extract of *Nauclea orientalis Linn.* has the highest measure of zone of inhibition against *S. aureus* compared to the other three concentrations. However, the control registered the highest zone of inhibition. The presence of zone of inhibition indicates that the crude alcoholic leaf extracts have anti-*Staphylococcal* activity.

Table 1. Measure of susceptibility and sensitivity of *S. aureus* to the different treatments in terms of zone of inhibition

Treatments	Zone of Inhibition in Millimeter (mm)			
	Replicate 1	Replicate 2	Replicate 3	Mean
25 %	11 mm	11 mm	11 mm	11 mm
50 %	12 mm	12.6 mm	12 mm	12.2 mm
75 %	15 mm	18 mm	11 mm	14.67 mm
100 %	20 mm	20 mm	14 mm	18 mm
Control Group	21 mm	22 mm	22 mm	21.67 mm

Table 2 shows the statistical significance of the zone of inhibitions. As reflected, the *P value* is 0.000921. This is lower than the 0.01 level of significance. This reflects a high significance and evidence that the null is rejected.

The Tukey's HSD test shown in table 3 also affirms that the 25% is not significantly different to 50% and 75% but is significantly different to 100% and to the control. 50% is not significantly different to 75% and 100% but significantly different to the control. 75% is not significantly different to 100% but is significantly different to the control. 100%, however, is not significantly different to the control treatment. This means that the 25% has a comparable anti-*staphylococcal* activity to the 50% and 75% but not with 100% and the control. Further, the effect of 50%, 75% and 100% extract are comparable with each other, however, only the 100% extract is comparable to the anti-*staphylococcal* activity of the control treatment.

The result of the test reflects that the crude alcoholic leaf extract of *Nauclea orientalis Linn.* has an anti-*staphylococcal* activity. The zone of inhibition that is formed becomes larger as the concentration increases.

Table 2. One-way ANOVA on the mean zone of inhibition of the treatments

Treatment	SS	df	MS	F	P
(Between Groups)	228.336	4	57.084	11.52	0.000921
Error	49.5733	10	4.9573		
Total	277.9093	14			

The observed bactericidal activity of *Nauclea orientalis Linn.* is due to the presence and action of the crude medicinal ingredients found in the matured leaves. (Raghavamma & Rao, 2010) Alkaloids are present in the plant which inhibits the growth of *S. aureus* by inhibiting its microtubules. Microtubules are essential in the bacterium's cytoskeleton, locomotion and adhesion. In addition, the leaves contains abundant amount of glycosides. This contributes to the bactericidal activity by binding with enzyme hyaluronidase of *S. aureus*. Tannins is a powerful phytochemical that facilitate the precipitation of bacterial proteins found in its wall. It also

promotes anti-inflammatory by binding with protein receptors resulting in the destruction of the bacteria.

Efficacy of the Formulated *Nauclea orientalis Linn.* Ointment

The 100% crude alcoholic leaf extract showed a comparable effect to the control in the earlier test. The extract was then used to formulate the ointment.

Table 3 shows the result of the test for efficacy of the formulated ointment. As shown, the *Nauclea orientalis Linn.* ointment registered 6 days duration of healing for replicate 1 and 7 days for replicates 2 and 3 with an average of 6.67 days. Erythromycin ointment showed 8 days duration of healing fro replicates 1 and 3 and 7 days for replicate 2 with a mean of 7.67 days. Lastly, natural healing registered 13 days, 14 days and 12 days duration of healing for replicates 1, 2 and 3 respectively with an average of 13 days.

Table 3. Duration of Healing

Treatment	Replicate 1	Replicate 2	Replicate 3	Mean
Nauclea orientalis Linn. Ointment	6	7	7	6.67
Erythromycin Ointment	8	7	8	7.67
Natural Healing	13	14	12	13

Table 4 shows that there is a significant difference among the treatments. Further, the Tukey's HSD revealed that the duration of healing of the formulated ointment of *Nauclea orientalis Linn.* is significantly different to natural healing but not significantly different to Erythromycin ointment. This means that the formulated ointment has comparable effect to Erythromycin ointment in terms of duration of healing of induced wounds infected with *S. aureus.*

The findings show that *Nauclea orientalis Linn.* has antibacterial and pro-repairing actions. This maybe be attributed to the presence of Tannins in the plants. According to Oana (2009), Tannins mode of action maybe related to their ability to inactivate microbial adhesion, enzymes, cell envelope transport protein, etc. External application promotes rapid healing of wounds and inflammation by acting with protein to form a protective layer such as the action of comfrey on wounds.

Table 4. One-way ANOVA on the Duration of Healing

Sources	SS	df	MS	F	P
Treatment (Between groups)	69.5556	2	34.7778	62.6	<0.0001
Error	3.3333	6	0.5556		
Total	72.8889	8			

The presence of alkaloids as revealed also defends the efficacy of the formulated ointment. Studies showed that some of the effects noted with alkaloids include powerful effects on phagocytosis and it increases T helper cells. Pentacyclic, which is a type of alkaloids, appear to even expedite wound healing. Many different types of alkaloids have displayed antimicrobial, antifungal, antitumor and anti inflammatory activities. (Nayak, Sandiford, & Maxwell, 2009)

Conclusion

Based from the findings, the researchers came up with the following conclusions:

1. The 100% crude alcoholic leaf extract of *Nauclea orientalis Linn.* had better anti-*staphylococcal* activity compared to the other concentrations but the control has the highest.
2. The anti-*staphylococcal* activity of 100 % crude alcoholic leaf extract of *Nauclea orientalis Linn.* is comparable to Erythromycin.
3. The formulated ointment from the 100% crude alcoholic leaf extract of *Nauclea orientalis Linn.* is comparable to the effect of Erythromycin ointment in wound healing. It can be used as a substitute for Erythromycin ointment in treating superficial wounds.

References:

Artizzu, N., Bongignore, L., Cottiglia, G., and Loy, G. (1999). Studies of the Diuretic and Antimicrobial Activity of Cyanodon dactylon Essential Oil. *Fitoterapia*. Vol. 66. pp. 493-496

Ballada, K. A., & Domondon, D. L. (2011). Phytochemical Screening and Antibacterial Potentials of Maoutia setosa Wedd., an Underutilized Plant in the Cordillera Administrative Region, Luzon, Philippines. In II International Symposium on Underutilized Plant Species: Crops for the Future-Beyond Food Security 979 (pp. 603- 610).

Barros, C. (1999). Antifungal Activity of Crude Leaf Extracts of Three Medicinal Plants:

Input to Science Education. Dissertation. Don Mariano Marcos Memorial State University, Agoo, La Union

Chambers, H. F. (2001). The changing epidemiology of Staphylococcus aureus?. Emerging infectious diseases, 7(2), 178.

Dasmariñas, C., Bayalan, H. K., Villa, M. P. I., & Landicho, D. L. (2008). HERBAL MEDICINE FOR ALTERNATIVE HEALTH CARE.

Edwards, R., & Harding, K. G. (2004). Bacteria and wound healing. Current opinion in infectious diseases, 17(2), 91-96.

Guerrero LM (1921). Medicinal Uses of Philippine Plants Bur. For. Bull., 22: 149-246.

Gwizdala, R. A. et al. (2011). Staphylococcus aureus colonization and infection among drug users: identification of hidden networks. *American journal of Public Health*, 101(7), 1268-1276.

Lowy, F. D. (1998). Staphylococcus aureus infections. *New England Journal of Medicine*, 339(8), 520-532.

National Institutes of Health. (nd). Staphylococcal infections. Medline Plus. http://www.nlm.nih.gov/medlineplus/staphylococcalinfections.html

Nayak, B. S., Sandiford, S., & Maxwell, A. (2009). Evaluation of the wound-healing activity of ethanolic extract of Morinda citrifolia L. leaf. *Evidence-Based Complementary and Alternative Medicine*, 6(3), 351 356.

Nita T. et al. (2002)Antibacterial Activity of Extracts Prepared from Tropical and Subtropical Plants on Methicillin Resistant Staphylococcus aureus. *Journal on Health Science*. Vol 48. pp. 273-276

Nolledo, J.N. (n.d.) TRADITIONAL AND ALTERNATIVE MEDICINE ACT OF 1997. post typhoon sky inc.

Oana, R. (2009). Anti-Staphylococcal Efficacy of Lantana camara Linn. (Bangbangsit). Thesis. Don Mariano Marcos Memorial State University Open System University, San Fernando City, La Union

Quisumbing, E. (1978). Medicinal Plants of the Philippines. Katha Publishing, Manila, p. 1225

Raghavamma, S. T. V., & Rao, N. R. (2010). In vitro evaluation of anthelmintic activity of Nauclea orientalis leaves. *Indian journal of pharmaceutical sciences*, 72(4), 520.

Tan, M. L., Querubin, M., & Rillorta, T. (1988). The integration of traditional medicine among community-based health programmes in the Philippines. *Journal of tropical pediatrics*, 34(2), 71-74.