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IN VITRO ANALYSIS OF THE ANTIBACTERIAL PROPERTIES OF *BASELLA RUBRA LINN.* (ALUGBATI) AND *PLUMIERA ACUMINATA* (KALACHUCHI) AGAINST MOST COMMON BACTERIAL WOUND ISOLATES AMONG PEDIATRIC PATIENTS

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Abstract

Objective: **To determine the antibacterial properties of *Basella rubra Linn.* (alugbati) and *Plumiera acuminata* (kalachuchi) against most common bacterial wound isolates among pediatric patients.**

Design: **Descriptive (retrospective) study and experimental study**

Setting: **Microbiology department of the Philippine General Hospital.**

Methods: **The first part of the study was the identification of the most common pediatric bacterial wound isolates and their antibiotic sensitivity patterns from January to June, 2000. The second part of the study was the in vitro analysis of the antibacterial properties of both aqueous and alcohol leaf extracts of alugbati and kalachuchi which was based on the Kirby Bauer method of disc susceptibility testing. Analysis of Variance (ANOVA) was used to analyze the difference between the mean zones of inhibition of the different treatments.**

Results: **The overall rank order of the five most common pathogens was *Staphylococcus aureus* (42%) > Group A β-hemolytic *Streptococcus* (15%) > *Pseudomonas aeruginosa* (12%) > *Escherichia coli* (8%) > *Klebsiella sp.* (5%). The study showed that the aqueous leaf extract of kalachuchi at two concentrations (50% and 100%) had in vitro antibacterial activity against *Staphylococcus aureus*, Group A β-hemolytic *Streptococcus*, and *Pseudomonas aeruginosa* and a significant difference between the mean zone diameter of inhibition for both concentrations was observed ($P<0.01$). Furthermore, there was increasing trend of zone of inhibition with increased concentration ($P<0.01$). Alugbati aqueous leaf extract also showed inhibitory effect on the growth of *Staphylococcus aureus* at 100% concentration only ($P<0.05$). Alcohol leaf extracts of both plants as well as the negative controls did not show any antibacterial activity against the test organisms.**

Conclusion: **Aqueous leaf extracts of kalachuchi exhibit in vitro antibacterial properties for S. aureus, Group A β-hemolytic *Streptococcus* and *P. aeruginosa*. Likewise, the aqueous leaf extract of alugbati also shows some inhibitory effect on the growth of *S. aureus* at 100% concentration but no inhibitory effect on *Streptococcus* and *P. aeruginosa*. The alcohol extracts of both plants and negative controls did not show any inhibitory effect on test organisms.**

Recommendation: **Further studies using the kalachuchi aqueous extract as an antibacterial wash/drug are warranted to determine its clinical usefulness.**

INTRODUCTION

Through the years, medicinal plants have been used by people who are living in the countryside in the treatment of various forms of diseases. However, rural health physicians would refrain from prescribing these herbal medicines unless scientific studies have been done to validate their effectiveness in medical treatment.

The use of medicinal plants as an alternative means of health care has been advocated by the Department of Health and World Health Organization. This is being promoted to help underprivileged people who cannot afford the cost of conventional antibiotics. Thus, the government and drug manufacturers are investing in researches and development of potential medicines from herbs and medicinal plants. However, research on herbal medicines should be based on the incidence of the disease in the locality, their availability, acceptability, and appropriate technology to determine their potency and safety.[1]

Health statistics in the country have shown that infectious diseases are among the top ten causes of morbidity among pediatric patients. Studies have shown that skin infections may account up to 6% of clinical visits in pediatric practice. The most frequent diagnoses included bacterial skin infections (36%), diaper dermatitis (16%), and atopic dermatitis (9%).[2] The organisms most commonly isolated from wound cultures are the gram-positive bacteria particularly *Staphylococcus aureus* and Group A β-hemolytic *Streptococci* (GABHS)[3]. Because of their tendency to produce systemic dissemination, efforts should be focused towards the eradication of the organisms either through the use of conventional antibiotics or possibly through the use of medicinal plants with antibacterial properties.

Keywords: Kalachuchi, *Plumiera acuminata*, Alugbati, *Basella rubra*, wound infections
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REVIEW OF LITERATURE

Medicinal plants in the Philippines describing their folkloric uses had been published as early as 1951 by Quisumbing (Medicinal Plants of the Philippines).[4]

Basella rubra commonly known as **alugbati** is found in settled and cultivated areas, in hedges throughout the Philippines. This is a succulent, branched, smooth, twining, herbaceous vine, reaching a length of several meters. The stems are green or purplish. The leaves are somewhat fleshy, ovate, or heart-shaped, 5 to 12 centimeters in length, stalked, tapering to a pointed tip, and cordate at the base. It is a very common and popular leafy vegetable and is much used in stews. The plant is mucilaginous when cooked. It was reported that it is an excellent source of calcium and iron.[5] Furthermore, it is a good source of vitamins A, B and C.[6]

The roots are applied as a rubefacient, and as a poultice to reduce local swellings; the sap is used to anoint any part of the body affected by acne to diminish the irritation.[7] Also is used as a mild laxative.[8] The leaves are reduced to a pulp and applied to boils, ulcers and abscesses. The juice of the leaves is thought to be useful in catarrhal conditions among children. The mucilaginous fluid obtained from the leaves and tender stalks of the plant is a popular remedy for habitual headaches.[9]

Plumiera acuminata (**kalachuchi**) is generally planted for ornamental purposes. This is a small, deciduous tree, 3 to 7 meters in height, with a crooked trunk, thick, fleshy branches with abundance of sticky milky juice. The branches are swollen and leafy at their tips. The leaves are alternate, oblong, 20 to 40 centimeters long, pointed at both ends, and spirally arranged at the ends of the branches. The flowers are supposed to be the source of perfume known as "Frangipani".[10]

The bark contains a bitter glucoside called plumierid. This contains resins, caoutchoue and calcium salts of plumieric acid: cerotinic acid and lupeol, and the leaves contain volatile oil.[11] Plumierid is a non-toxic, non-irritant to the eye, and with no effect on the respiration and circulation even after intravenous injection.[12] The most important effect of plumierid observed in animals and confirmed in man when administered by mouth was catharsis. A decoction of bark is used as a purgative and febrifuge.[7]

The heated leaves are applied as a poultice to boils, swellings and eruptions of the soles of the feet. In infusion or as an extract, the leaves are reported to be effective cure for asthma. Alcohol leaf extract of *Plumiera sp.* was bioassayed using the micronucleus test and was found to have an antimutagenic activity.[13]

The medicinal values of *Basella rubra* and *Plumiera acuminata* have been described. However, their effectiveness in the eradication of organisms causing pyogenic dermatoses among children has not yet been thoroughly investigated.

OBJECTIVES

1. General

1.1. To determine the antibacterial properties of both aqueous and alcohol leaf extracts of *Basella rubra* and *Plumiera acuminata* against common bacterial wound isolates among pediatric patients of a tertiary care hospital.

2. Specific

2.1. To identify the most common bacterial wound isolates and their respective antibiotic sensitivity patterns among pediatric patients of the Philippine General Hospital from January to June, 2000 through a retrospective study.

2.2. To determine the antibacterial properties of **alugbati** and **kalachuchi** leaf extracts at two different concentrations—50% and 100% through Kirby Bauer method of disc susceptibility testing against the top three most common bacterial wound isolates.

2.3. To compare the antibacterial activity of the plant extracts against a positive (recommended antibiotic of choice) control and negative (distilled water) control.

METHODOLOGY

STAGE I Identification of the organisms isolated from wound discharge or aspirate specimen and their antibiotic sensitivity patterns among pediatric patients

Data were obtained from the wound culture and sensitivity logbook of the Bacteriology Section of the Department of Laboratories of UP-PGH from January to June, 2000. The top three most common bacterial wound isolates were used as test organisms for the second part of the study. Likewise, the antibiotic of choice identified for each test organism was used as a positive control in testing the antibacterial properties of the plant extracts.

STAGE II Testing of the antibacterial properties of the leaf extracts against the top three most common bacterial wound isolates identified in Stage I such as *Staphylococcus aureus*, Group A β-hemolytic *Streptococcus* and *Pseudomonas aeruginosa*.

This part of the study involves two phases:

PHASE I Crude Extraction of Selected Medicinal Plants

1. Aqueous Extraction Phase

Leaves of both **alugbati** and **kalachuchi** were separately harvested in the morning, thoroughly washed, comminuted, and weighed to obtain 100 grams. The leaves were then blenderized until a fine consistency was obtained. Enough water was added until the compound was sufficiently wet (the volume of water in ml. and weight of the material having a ratio of 1:1). The blenderized plant extracts were filtered using a cloth bag, then ten times through a filter paper. The filtrates were then centrifuged at 3,000 rpm for ten minutes. Filter sterilization of the extracts using 0.45 µm millipore filter paper was done to remove any contaminant bacteria. The filtrates were then transferred to sterile Florence flasks and placed inside the freezer until frozen.

Lyophilization of the extracts then followed until a powder form was obtained. The lyophilized extracts were then weighed and dissolved in distilled water with 1:1 ratio and 1:2 ratio to obtain a 100% and 50% concentration respectively. The extracts were then ready for bacteriologic assay.

2. Alcohol Extraction Phase

Leaves of both **alugbati** and **kalachuchi** were separately harvested in the morning, thoroughly washed and allowed to sun dry, then were grinded. Sixty grams of the ground dried plant materials were then soaked in 60 ml. of 80% ethyl alcohol for 48 hours. After filtration through a cloth bag and ten times through the filter paper, the plant extracts underwent rotavaporization up to 50°C to remove the alcohol used to extract the plant components. The resultant gel forms were then weighed and dissolved in distilled water with a ratio of 1:1 and 1:2 to obtain 100% and 50% concentration respectively.

PHASE II Bacteriologic Assay

1. Preparation of Agar Plates

Overnight broths of *Staphylococcus aureus* ATCC 25923, Group A β-hemolytic *Streptococcus* ATCC 21547 and *Pseudomonas aeruginosa* ATCC 27853 were used as test organisms. The broths were standardized by dilution using a nutrient broth to a final density of 0.5 McFarland units= 1.5×10^8 cells/ml. Mueller Hinton media were used as culture plates. Inoculation of the test organism was done using the streak plate method. A sterile cotton swab was dipped in the inoculum and the surface of the plate was streaked three times for each time the plate was rotated 60° to ensure an even distribution of the inoculum.

2. Preparation of the Controls

A positive control was defined as the recommended antibiotic of choice for the isolated test organism which was based on the sensitivity patterns obtained from Stage I of the study. The antibiotics used were the commercially prepared sensitivity discs. They were as follows: (a) *Staphylococcus aureus* – Oxacillin (1µg); (b) Grp A β-hemolytic *Streptococcus* – Penicillin G (10 units); (c) *Pseudomonas aeruginosa* – Ceftazidime (30 µg). Distilled water was used as a negative control.

3. Application of Discs

Commercially available paper discs with a diameter of 6 mm and volume of 0.325 ml. were used. The disc was dipped in each of the extracts until fully soaked. Excess liquid was removed from the disc by placing it over a sterile filter paper. Discs containing the different plant extract preparations at two concentrations (50% and 100%) together with the antibiotics and the negative controls were placed in each petri dish containing the test organism. The petri dishes were incubated at a temperature of 35°C- 37°C for 18 hours overnight in an inverted position. At the end of the incubation period, zone of inhibition was observed in each plate and the diameter was measured using a sliding caliper and a magnifying glass.

Analysis of Variance (ANOVA) was used to analyze the difference between the mean zones of inhibition of the different treatments.

RESULTS AND DISCUSSION:

Stage I Identification of organisms isolated from wound discharge or aspirate specimens and their antibiotic sensitivity patterns among pediatric patients

For a period of six months (January to June, 2000), there were 199 wound discharge/aspirate specimens included in the study. A total number of 227 isolates was recorded with an average of one isolate per specimen. Ten (5%) of the wound cultures showed two bacterial isolates per specimen. In this study, *Staphylococcus aureus* (42%) was still the most common bacterial wound isolate noted among pediatric patients. This was followed by Group A β-*hemolytic Streptococcus* (15%), *Pseudomonas aeruginosa* (12%), *Escherichia coli* (8%), *Klebsiella sp.* (5%) and others (18%), which include *Enterobacter cloacae*, *Hafnia alvei*, *Enterobacter aerogenes*, *Proteus mirabilis*, *Acinetobacter anitratus*, *Serratia marcescens*, *Pseudomonas putida*, *Staphylococcus epidermidis* and *Streptococcus viridans* as shown in Figure 1.

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Base on the results of the study, it was found out that ***kalachuchi*** aqueous extract demonstrated greater inhibitory effect on the test organisms compared with ***alugbati***. The most practical application of this study was that the extracts may be tested *in vivo* as a handwash, antiseptic, or wound cleanser to test their clinical antibacterial properties.

The study utilized two methods of plant extraction — aqueous and alcohol extractions. The purpose of this was to determine the most appropriate method in obtaining the crude plant extract suitable for bacteriologic assay. Therefore, it was the limitation of this study that the plant extract used was not fractionated and purified and thus, the active component with antibacterial activity is yet to be isolated and identified.

CONCLUSION

The most common bacterial wound isolates among pediatric patients are: *Staphylococcus aureus*, Group A β-hemolytic *Streptococcus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella sp.* The recommended antibiotic of choice based on this study for the top three most common organisms are: Oxacillin - *Staphylococcus aureus*; Penicillin G - Group A β-hemolytic *Streptococcus*, and Ceftazidime - *Pseudomonas aeruginosa.*

Data from this experimental study show that aqueous leaf extracts of ***kalachuchi*** at two concentrations—50% and 100% exhibit *in vitro* antibacterial activities for *S. aureus*, Group A β-hemolytic *Streptococcus* and *P. aeruginosa*, although the inhibitory effects were less compared with the positive antibiotic controls. There is also increasing trend of zones of inhibition with increased concentration. Likewise, the aqueous leaf extract of ***alugbati*** shows inhibitory effect on the growth of *S. aureus*. The alcohol leaf extract of both plants and the negative controls did not show any antibacterial activity against the test organisms.

RECOMMENDATIONS

Based on the findings of this study, we recommend that:

1. Other methods of extraction should be tested since the alcohol extraction process might have destroyed the active components of the plant causing no inhibitory effect on the growth of the test organism.
2. The active components of the plant with antibacterial properties should be properly isolated and identified.
3. *In vivo* analysis of the antibacterial properties of **kalachuchi** aqeous leaf extract should be performed.
4. Other parts of the plant should be tested and compared regarding their antibacterial potentials.

REFERENCES

1. Cortes-Maramba, NP et al. Guidebook on the proper use of medicinal plants. NSTA-PCHRD, Taguig, Manila, 1982.
2. Hayden, GF. Skin diseases encountered in pediatric clinic: a one year prospective study. American Journal of Diseases of Children. 139(1): 36-8, 1985.
3. Dajani, AS Ferrieri, P and Wanamaker, LW. Natural history of impetigo II. Etiologic agents and bacterial interactions. Journal of Pediatrics, 76:676. 1970.
4. Quisumbing, E. Medicinal plants of the Philippines. Quezon City, Philippines: Katha Publishing House Co., 1978.
5. Maranon, J. Nutritive mineral value of Philippine food plants (calcium, phosphorous, and iron contents. Philippine Journal of Science, 1935.
6. Hermano, AJ and Sepulveda G. The vitamin contents of Philippine foods III: Vitamin B in various fruits and vegetables. Phil Jour Sci 54:61-73, 1938.
7. Guerrero, Leon Ma. Medicinal uses of Philippine plants. Philippine Bureau of Forestry Bulletin 760-787, 1921.
8. Nadkarni, K.M. Indian Materia Medica. Bombay, India. 1142, 1956.
9. Stuart, G.A. Chinese Materia Medica. Shanghai. 558. 1911.
10. Bacon, RF Philippine terpines and essential oils III. Philippine Journal of Science A4: 93-132. 1909.
11. Wehmer, RC. Die Pflanzenstoffe. JENA. 1:640. 1929.
12. Garcia, F and Santos, AC. Chemical and pharmacological studies of plumierid. Rev Fil Med Farm. 22:254-265, 1931.
13. Guevarra, A. et al. Isolation and characterization of an antimutagen from Plumiera acutifolia Ptor. 7th Asian symposium on medicinal plants, spices and other natural products. PTP: 18. 1992.

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14. Jawetz et al. Medical Microbiology, 18th ed. San Mateo, California: Appleton and Lange, 1989.
15. Lagaya, AT et al. In vitro analysis of the antibacterial properties of medicinal plants against commonly isolated gram-positive and gram-negative organisms of acute upper respiratory tract infection among pediatric patients. Philippine Journal of Pediatrics 117(3):231-235, 1988.
16. Power, DA. and McCuen, PJ. Manual of Laboratory Procedures. Becton Dickinson Microbiology, 5th ed. Cockeysville, Maryland, 1988.

RISK FACTORS AND MICROBIOLOGY OF NOSOCOMIAL INFECTION AMONG NICU PATIENTS AT A TERTIARY HOSPITAL

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Abstract

A retrospective study was done on the risk factors and microbiology of nosocomial infection among NICU patients at the University of the East Ramon Magsaysay Memorial Medical Center. The study period was done from January 1, 1995 to August 31, 2000. Newborns were divided into 2 groups: those who developed and those who did not develop nosocomial infections. Based on the results, nosocomial infection was proven to be a significant cause of morbidity and mortality. The number of antibiotics used and duration of hospital stay are the risk factors found to be predictive of nosocomial infections. The predominant organisms isolated from all sites were mainly gram negative pathogens. Increased resistance of those organisms to aminoglycosides, cephalosporins, and extended spectrum penicillins was evident. The emergence of candidemia in the last two years of the study period was noted.

INTRODUCTION

Great strides in pharmacology and technology in the care of the newborn through the establishment of neonatal intensive care units made possible the survival of very small prematures. However, with this is noted a parallel increase in the incidence of hospital acquired infection. Although the number of hospital beds allotted to NICU is small, a large percentage of patients are infected with hospital acquired organisms. This translates to extended hospital stay, added hospitalization cost and eventually increased mortality rate.

It is the purpose of this paper to determine the incidence and risk factors associated with nosocomial infection in NICU together with the identification of etiologic agents and current management.

This knowledge will enable us to plan on surveillance of those with hospital acquired infection.

Keywords: Nosocomial infection, Neonatal intensive care unit
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MATERIALS AND METHOD

Medical records of all newborn admitted at the NICU of University of the East Ramon Magsaysay Memorial Medical Center, a tertiary teaching hospital from January 1995 to August 31, 2000 were reviewed.

Newborns were divided into 2 groups:

Those who developed and those who did not develop nosocomial infections

Factors such as the following were determined

Age of gestation, sex, weight, presence of fever, primary disease, procedure done, duration of stay, onset of nosocomial infection, number of antibiotic used and outcome.

Newborns with the following were excluded:

History of PROM, history of maternal infection, positive initial cultures and those admitted in other units for more than 48 hours or discharge from other units within 48 hours.

Culture and sensitivity results were likewise recorded.

Definition of terms

Nosocomial infection is defined as a localized or systemic condition that developed after admission to the hospital as an adverse reaction to the presence of an infectious agent or its toxins which is not present or incubating at the time of admission.

Nosocomial infections were considered ICU associated, if they developed in the ICU 48 hours after admission or within 48 hours of discharge from the unit, unless the clinical evidence strongly suggested otherwise.

Statistical analysis was done using the Chi square test, student's test and stepwise logistic regression to determine which among variables were significant as risk factors for nosocomial infection.