Hemostatic Activity of Saba Banana (*Musa sapientum Linn. Var. compressa*)
Peel Extract on Sparague Dawley Rats

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Abstract

Cardava banana (*Musa sapientum Linn. Var. compressa*) is one of the most important banana cultivars in the Philippines. The peel is usually wasted and poses an environmental concern if not utilize properly. Recent studies reported that unripe banana peel contains tannins and leucocyanidin, which can accelerate and aid wound healing. The objectives of the study are to evaluate the anti-hemorrhagic effect of the different concentrations of *Musa sapientum Linn. Var. compressa* extract using Duke’s modified bleeding time before and after application of the MSVC extract in different concentrations; 100%, 75% and 50%. Extract of the unripe MSVC was taken from 24 kilograms dried banana peel that went through a drying process at 50 degrees Celsius and 10% relative humidity (%RH) using a Multi-Commodity Heat-Pump Dryer (MCHPD). All the rats were sedated with Zoletil 50. Rats were precisely wounded intraorally at the buccal vestibule of the lower central incisors using surgical blade number 15. The depth and length of the wounds were controlled and measured by stopping at bone depth upon incision of about 0.5 centimeters. The wounded area was pat dried every 30 seconds, then the bleeding time was recorded every after pat drying until the bleeding stops. The results were computed statistically using ANOVA and Post Hoc test which conclusively showed that 50.0% extract concentration has the most significant difference with a p-value of 0.009 as compared with other concentrations.

Keywords: hemostatic activity; *Musa sapientum Linn. Var. Compressa*; banana peel extract; Sprague dawley rats
Introduction

Organic products are proven to be useful and effective in this era; wherein, numerous studies are funded and conducted to prove and discover their medical and healing potentials. In the development of therapeutic agents for infectious diseases between 1981 and 2006, more than 70% of the agents developed were derived from natural products (Newman and Cragg, 2007). The high chemical diversity and biochemical specificity possessed by naturally occurring compounds, together with their other molecular properties, make them very good candidates for discovery and development of drugs (Koehn and Carter, 2005). In the Philippines, one good source of a natural product is banana. Banana (*Musa sapientum* Linn. *Var. Compressa*) is no doubt a delicious and nutritious fruit. In fact, banana one of the most important cultivars in the Philippines (Safepac Corporation Davao, 2015) where *Musa sapientum* Linn. *Var. Compressa* (*MSVC*) are very common. It is almost found everywhere in the country. Additionally, it does not only yield fruits seasonally. It can yield banana fruits any season of the year, which makes it affordable and available in most of the cities and towns in the country. *Musa sapientum* Linn. *Var. Compressa* (*MSVC*) is a great source of living. It can be eaten raw or cooked into various traditional Filipino desserts or dishes like *maruya*, *turon*, mixed with *halo-halo*, *ginanggang*, *minatamis*, *sinapot*, or boiled and eaten with *ginamos*(fermented fish), and is used as one of the recipes of *nilaga* (Safepac Corporation Davao, 2015). However, despite its use for food purposes its peels is usually wasted and left at the trash. On the other hand, bleeding is an unavoidable sequela of oral surgery. Simple extractions, odontectomy procedures, bone reduction, and even accidental slip of dental instruments can all cause bleeding. Bleeding during the surgical procedure can block the visibility and access of the dentist that can further delay or sometimes complicate a dental procedure. To date, some measures are done to control bleeding during and after an oral surgical procedure. Such measures are the following: (1) applying pressure to the wound, (2) injecting vasoconstrictors on the surrounding area of the wound, and (3) electro cauterization. Post surgically, surgeons usually prescribe analgesics and antibiotics only for comfort and aid on healing. If hemostasis can be hasten, wounds can also heal faster and be less prone to complication. There are recent studies showing that unripe banana peel contains leucocyanidin, a flavonoid that induces cell proliferation, accelerating the healing of skin wounds. It also contains tannins, which is essential in the wound healing process, repair and maintenance of cartilage, bones, and teeth. It is an antioxidant and is non-toxic (Soliduum, 2011). The fresh sap which is also used as local hemostatic for the treatment of external wounds has been reported by other authors for its hemostatic and wound healing. Thus, in Brazil, its sap is used for treatment of bleeding (Albuquerque, 2007). In support to this, based in studies conducted it was proven that banana sap has hemostatic potential; the researchers would want to test this discovery further by analyzing the hemostatic effect of the unripe banana peel on Sparague Dawley rats using Duke’s modified bleeding time.
Objectives of the Study

General

To develop different concentrations of extract and test in vivo the hemostatic effect of the *Musa sapientum Linn. Var. compressa* (MSVC) extract. Further, it aims to: (1) to evaluate the anti-hemorrhagic effect of the different concentrations of *Musa sapientum Linn. Var. Compressa* (MSVC) extract using Duke’s modified bleeding time and; (2) to determine whether there is a significant difference between the bleeding time before and after application of the *Musa sapientum Linn. Var. Compressa* (MSVC) extract in different concentrations; 100%, 75% and 50%.

Review of Literature

The *Musa sapientum Linn. Var. Compressa* scientific classification: (Carl Linnaeus, 1753)

<table>
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<th>Kingdom</th>
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<tr>
<td>Order</td>
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<tr>
<td>Scientific Name</td>
<td><em>Musa sapientum Linn. Var. Compressa</em></td>
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<td>Common Name</td>
<td>Cardava banana, Saba banana</td>
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Musa, a plant genus of extraordinary significance to human societies produces the fourth most important food in the world today (after rice, wheat, and maize), bananas and plantains. Musa species grow in a wide range of environments and have varied human uses, ranging from the edible bananas and plantains of the tropics to cold-hardy fiber and ornamental plants. They have been a staple of the human diet since the dawn of recorded history. These large, perennial herbs, 2–9 m (6.6–30 ft) in height, evolved in Southeast Asia, New Guinea, and the Indian subcontinent, developing in modern times secondary loci of genetic diversity in Africa, Latin America, and the Pacific (Nelson, 2011).

**Musa sapientum Linn. Var. Compressa**

MSVC is a triploid hybrid (ABB) banana cultivar originating from the Philippines. It is primarily a cooking banana though it can also be eaten raw. It is one of the most important banana varieties in Philippine cuisine. It is also sometimes known as the cardava banana, though the latter name may be more correctly applied to a very similar cultivar which is also classified within the Saba subgroup (Dela Cruz, 2008).
**Active Constituents Musa sapientum Linn. Var. Compressa (MSVC) Peel**

The peel wastes from Saba may contain the same valuable components generally found in banana flesh. These valuable substances may be used to formulate preparations with pharmacologic/medicinal, nutritive, and energy values. *Musa sapientum Linn. Var. Compressa* or Saba is found in cultivation throughout the Philippines. Its fruit contains carbohydrates that exceed 25%. It is a good source of vitamin A, fair source of vitamin B, and good source of vitamin C. It contains potash and soda, chloride of potassium, alkaline phosphates with a little sulfate, lime, and silica among others. Peels of Saba were analyzed using phytochemical tests. From the results of the tests Saba peel extracts showed to be acidic; further it contains reducing substances, tannins, mucins, proteins, alkaloids, saponins and flavonoids. Tannins are used in the treatment of minor burns. It promotes the formation of a firm eschar. It has been used in the treatment of bedsores, weeping ulcers, ingrown toe nails, and ivy poisoning. It may treat toothache, cancer, and cold sores. It may be used as an internal antidote to precipitate toxic alkaloids and to harden the surface of the gastrointestinal mucosa and its mucous layers. Tannins are used as astringents and protein precipitants; moreover, may be used as an antimicrobial. Bananas are said to be good sources of vitamin C. Ascorbic acid is an essential nutrient for humans. Its regular intake is known to increase the immune system and prevent common diseases such as colds. It is valuable to growth and repair of tissues in all parts of the body and even in the formation of collagen, tendons, ligaments and blood vessels. It is essential in the wound healing process, repair and maintenance of cartilage, bones, and teeth. It is an antioxidant and it is non-toxic (Soliduum, 2011).

**Medicinal Uses of Musa sapientum**

*Musa sapientum* fruits have been reported to prevent anemia by stimulating the production of hemoglobin in the blood. Its role to regulate blood pressure has been associated with the high content of potassium. Banana helps in solving the problem of constipation without necessary resorting to laxatives. It has been reported that banana can cure heart burns, stress, strokes, ulcers and many other ailments. The peels have been
reported to be useful in making banana charcoal, an alternative source of cooking fuel in Kampala. Also, the peels in conjunction with other substances create a liniment for reducing the acuteness of the arthritis aches and pains.

**Hemostatic Potential of Banana Peel Extract**

The pseudo-stem of the banana plant and unripe banana peels contain copper, zinc, sodium, potassium, calcium, phosphorus, and iron; further, the fruit contains antioxidants, including vitamins C and E, and beta carotene. Unripe banana extract promotes increased incorporation of thymidine into cellular DNA, which enhances cell proliferation. Unripe banana peel contains leucocyanidin, a flavonoid that induces cell proliferation, accelerating the healing of skin wounds. The pulp and peel of unripe banana have been used in the treatment of peptic ulcers in humans. Studies with rats have shown the efficacy of unripe banana in the prevention and treatment of peptic ulcers. The active agent in unripe bananas is water soluble and becomes inactive in ripe bananas. The fresh sap is also used as local hemostatic for the treatment of external wounds and this plant has been reported by other authors for its hemostatic and wound healing. Thus, in Brazil, its sap is used for treatment of bleeding (Albuquerque, 2007). The traditional use of sap of *M. sapientum* in the treatment of bleeding is warranted. Its mechanism of action is vasoconstriction and create the formation of a protein network that serves as a focal point to cell aggregation which leads to stop of bleeding (Klotoè; Dougnon, 2010).

**Chemical Composition of *Musa sapientum***

The result of mineral content shows the concentration of potassium to be highest Sa (78.10mg/g). The concentration (mg/100g) of calcium, sodium, iron, and manganese were 19.20, 24.30, 0.61 and 76.20 respectively. The result agrees with Akinyoye (2011) that banana fruit has high concentration of potassium. The appreciable high content of potassium signifies that if the peel is taken, it will help in the regulation of body fluids and maintain normal blood pressure. It will also help in controlling kidney failure, heart oddities, and respiratory flaw. Iron concentration was lowest, although, much lower values had been reported for the fruit. Iron carries oxygen to the cells and is necessary for the production of energy, synthesis of collagen, and the proper functioning of the immune system. Its low concentration implies that banana peel is an idyllic source of iron since its excess is implicated in abnormal functioning of the immune system, cell growth and the heart. Manganese known to aid formation of skeletal and cartilage was also found to be high (76.20mg/100g). Manganese dearth is scarce but could affect glucose tolerance, normal reproductive, skeletal and cartilage formation. The concentrations of the non-essential minerals like bromine, rubidium, strontium, zirconium and niobium were found to range between 0.21 – 0.02 mg/100g. The result implies that banana peel contained very low concentrations of the non-essential minerals. The levels of tannins in green bananas range from 122.6 mg to 241.4 mg. As bananas ripen, the tannin content decreases and becomes part of the pulp. Current research indicates that condensed tannins are also in the cell walls, which are a suitable source of natural antioxidants that are biologically accessible in the stomach. Tannins have also been reported to exert other physiological effects, such as accelerating blood clotting, reducing blood pressure,
decreasing the serum lipid level, producing liver necrosis, and modulating immune responses. Tannins may be employed medicinally in antidiarrheal, hemostatic, and anti-hemorrhoidal compounds. The anti-inflammatory effects of tannins help control all indications of gastritis, esophagitis, enteritis, and irritating bowel disorders. Diarrhea is also treated with an effective astringent medicine that does not stop the flow of the disturbing substance in the stomach; rather, it controls the irritation in the small intestine (Cheng, 2002). Tannins not only heal burns and stop bleeding, but they also stop infection while they continue to heal the wound internally. The ability of tannins to form a protective layer over the exposed tissue keeps the wound from being infected even more. Tannins are also beneficial when applied to the mucosal lining of the mouth (Stephane, 2004). Tannins can also be effective in protecting the kidneys. Tannins have been used for immediate relief of sore throats, diarrhea, dysentery, hemorrhage, fatigue, and skin ulcers. Tannins can cause regression of tumors that are already present in tissue, but if used excessively over time, they can cause tumors in healthy tissue. They have been also reported to have anti-viral (Lin, 2004), antibacterial (Akiyama, 2001), and anti-parasitic effects (Kolodziej, 2005).

**Clotting and Healing in the Oral Cavity**

On the surface of these activated platelets, many different clotting factors work together in a series of complex chemical reactions (known as the coagulation cascade) to form a fibrin clot. The clot acts like a mesh to stop the bleeding. Coagulation factors circulate in the blood in an inactive form. The healing of skin wounds is characterized by several, partly overlapping, stages; immediately after the wound is created, blood vessels constrict and blood clotting begins. Both processes are aimed to limit further blood loss. This is followed by the inflammatory phase, during which macrophages and other inflammatory cells remove bacteria and necrotic cell debris. At the same time, the inflammatory cells secrete factors that stimulate cell division and migration of cells, such as epithelial cells and fibroblasts. The proliferative phase. In this phase many regenerative processes take place, including angiogenesis, deposition of a new collagen matrix, and formation of granulation tissue. The wound is gradually covered with epithelial cells and wound contraction may occur.

**Bleeding Time**

A bleeding time test determines how quickly your blood clots to stop bleeding. The test involves making small, superficial cuts on your skin. They are similar to light scratches. The test is a basic assessment of how well your blood platelets work - form clots. Platelets are tiny cell fragments that circulate in your blood. They are the first cells to react to a blood vessel injury. They seal off the wound to prevent more blood from escaping.

**Measurement of Bleeding Time**

Duke’s method: Sterilize the fingertip using rectified spirit and allow to dry. Make a sufficiently deep prick using a sterile lancet, so that blood comes out freely
without squeezing. Note the time (start the stop-watch) when bleeding starts. Mop the blood by touching the fingertip with a filter paper. This is repeated every 15 seconds, each time using a fresh portion of the filter paper, till bleeding stops. Note the time (stop the stop-watch). It is seen that the blood stains on the filter paper get smaller to disappear finally when bleeding stops. Normal bleeding time (Duke’s method) is up to 4 minutes.

Materials and Methods

Research Design

Experimental study design was used to test the hypothesis by performing an in vivo study on Sprague Dawley rats. The laboratory rat species were divided into two main groups: the control group and the treatment group. The treatment group was further divided into three subgroups: Treatment 1 (T1) – 100% MSVC peel extract, Treatment 2 (T2) – 75% MSVC peel extract and, Treatment 3 (T3) – 50% MSVC peel extract.

Materials

I. Collection and Preparation of MSVC Peel
   1. 80 kilograms unripe MSVC fruit
   2. 24 kilograms of peel obtained from the MSVC fruit
   3. 4.2 kilograms of dried MSVC peel
   4. Weighing Scale - was used to obtain the weight of MSVC peels accurately.
   5. Scissors- was used to cut the banana peel into smaller partitions.
   6. Stainless Steel Knife - was used to separate the fruit from the peel.
   7. Multi Commodity Heat Pump Dryer (MCHPD) - this device was used to dry the MSVC peel.
   8. Air Tight Plastic Bags- these were used to pack the peel for easy transportation.

II. Collection and Preparation of MSVC Peel Extract at Different Concentrations
   1. 2 Kilograms of Dried MSVC Peel
   2. 8 Liters of 95% Ethanol
   3. Rotary Evaporator – used to filter the MSVC mixture
   4. Willey Mill – was used for pulverizing the dried MSVC peel.
   5. Distilled Water – was used to dilute the MSVC extract at different concentrations.

III. Preparation of In Vivo Testing
   1. 11 Female Sparague Rats with average weight of 110 grams each
   2. 11 Pieces of Surgical Blade Number 15- were used to create incisions on the rats.
   3. Zoletil 50 - a sedative.
   4. 10ml/cc Disposable Syringe 21 Gauge- was used to dispense the MSVC extract.
   5. Cotton Pliers – were used hold the cotton pellets.
   6. Adson Tissue Forceps – were used to retract the tissues.
7. Blade Holder – was used to hold the blade.
8. Cotton Pellets – were used to pat dry the wound.

IV. Sterilization Protocol
1. Scrub Suits
2. Slippers
3. Latex Free Gloves
4. Face Masks
5. Head Caps

Methods

I. Collection and Preparation of MSVC Peel

The unripe fresh MSVC fruits were directly harvested from Biga, Silang, Cavite and delivered at Adventist University of the Philippines Campus right after the collection. 80 kilograms of MSVC fruits were peeled off using stainless steel knives. The peels were strictly separated from the other parts of the fruit collecting only the peels. The collected peels weighed 24 kilograms as needed to obtain enough extract. The peels were cut in its desired size (approximately 1 inch in length and width) using scissors. After the collection and preparation, the MSVC peel were stored in air tight plastic bags and were brought to the Nutrition and Dietetics Department at Adventist University of the Philippines for drying.

Figure 2. Collection and Preparation of MSVC Peel
II. Drying of the MSVC Peel

The 24 kilograms of total MSVC peels were equally divided on 21 trays of the Multi Commodity Heat Pump Dryer (MCHPD) (Taclan, 2012) which weighed 1.11 kilograms each. Every tray was loaded with 1.14 kilograms of MSVC peels. For 16 hours, the MSVC peel were dried at 50 degrees Celsius and 10% relative humidity (RH). After drying, the MSVC peel weighed 0.20 kilograms per tray obtaining a total of 4.2 kilograms all in all. Through the Multi Commodity Heat Pump Dryer (MCHPD) every needed constituent from the MSVC peels were retained. Then, the dried MSVC peel were sealed in air-tight plastic bags to avoid contamination of other materials and were kept under moisture-free conditions for the next procedure which is extraction.

![Figure 3. Drying of MSVC Peel](image)

II. Extraction of the MSVC Peel

The extraction of the MSVC peel was made possible by the help of the Chemical and Energy Division of the Department of Science and Technology. Every part of the extraction procedure was done carefully.

First, 2.0 kilograms of the dried MSVC peels were pulverized using Wiley Mill and soaked in 8.0 L of 95% Ethyl Alcohol for 48 hours. The mixture was filtered and the filtrate obtained was concentrated using rotary evaporator at 60 degrees Celsius under
vacuum for 4 hours. The concentrated extract was further evaporated using water bath at 60 degrees Celsius to obtain semi-solid extract. The crude extraction of 2.0 kilograms of dried MSVC peels produced 7.0 L ethanolic extract. Concentration of the filtrate yielded 23.0 grams of semi-solid extract. Then, extracts were divided into three concentrations, 100% pure MSVC peel extract, 75% diluted with distilled water, and 50% diluted with distilled water.

![Figure 4. MSVC Peel Extract at Different Concentrations](image)

**IV. Evaluation of the Anti-hemorrhagic Effect at Different Concentrations**

This procedure is based on the Standard Laboratory Procedures of the Industrial Technology Division of the Department of Science and Technology. It was done by the researchers with the help and guidance of the staff of the said department.

**Sterilization Technique**

Every instrument used in the procedure was sterilized. The disposables such as surgical blades, syringes, and cotton pellets were bought sterilized and were just opened during the procedure. Other instruments such as cotton pliers, Adson forceps, and blade holders were autoclaved at Adventist University of the Philippines in the College of Dentistry prior to the procedure and were placed on sterilizing pouches. Before entering on the laboratory room, the staff and the researchers wore their caps, masks, gowns, slippers, and gloves to maintain the sterility on the area.
Population Sample

In this study, laboratory rats were used with the following inclusion criteria:

1. All rats are of the same species (female Sparague Dawley).
2. All rats have the weight within the range 69-122 grams.
3. All rats were acclimatized for 7 days.

Evaluation of the Anti-hemorrhagic Effect of MSVC using Duke’s Modified Bleeding Time

In order to determine the hemostatic potential of the MSVC peel extract, 11 laboratory rats were used in the experiment. Laboratory animals were acclimatized for seven (7) days. For dose/sample administration, animals were divided into two (2) major groups: Group 1- Control Group; Group 2- Treatment Group at 100%, 75% and 50% sample extract.

All the rats were sedated with Zoletil 50. Rats were precisely wounded intraorally at the buccal vestibule of the lower central incisors using surgical blade number 15. The depth and length of the wounds were controlled and measured by stopping at bone depth upon incision of about 0.5 centimeters. The wounded area was pat dried every 30 seconds, then the bleeding time was recorded every after pat drying until the bleeding stops. In group 1, no substance was applied on the wound of the test animal and was only
observed for bleeding time while in group 2, sample extracts (in different concentrations) was administered to the incised wound of the animal right after the incision using disposable syringe and was also observed for bleeding time. Animal weights were also determined and recorded. All animals used in the study were humanely euthanized by the in-house veterinarian and properly disposed. The mean differences were taken from the results of the wound sites and were compared to see if there was a significant difference with the bleeding time before and after applying the MSVC peel extract.

Figure 7. Sedation and Incision of the Rats
Figure 8 Flow of the Procedure

Statistical Treatment and Analysis of Data

For the statistical treatment and analysis of the data, all the data were entered in Statistical Package for Social Sciences (SPSS). T-test and ANOVA were used to compare the groups. All data were interpreted based on the $p=0.05$ significance level.
Summary, Conclusions, and Recommendations

Summary

Based on statistical analysis done on the control group vs 100%, 75% and 50% extract groups, the application of the MSVC peel extract showed significant difference against the group with no application. Also, based on the result of the hemostatic analysis, 50% MSVC extract yielded the shortest bleeding time result among the three extract concentrations. It also appeared to be the most statistically significant using a Post Hoc Test. Furthermore, based on the result of the hemostatic analysis, the bleeding time of rats is comparable to the range of bleeding time in humans based on studies.

Conclusions

In the study, it is shown that Musa sapientum Linn. Var. Compressa exhibits an anti-hemorrhagic effect on Sparague rats tested using modified bleeding time. It exhibits significant anti-hemorrhagic effect at different level of concentrations. The 50% concentration of Musa sapientum Linn. Var. Compressa exhibits the shortest mean bleeding time results and it was also the most statistically significant. Hence, the study revealed a significant difference between the control group and the Musa sapientum Linn. Var. Compressa extract treated groups using modified bleeding time. Furthermore, the different levels of concentrations of MSVC extract specifically 100%,75%,and 50% exhibited varying bleeding time results. Therefore, the researchers reject the hypotheses that there is no anti-hemorrhagic effect of the different concentration of MSVC extract using modified bleeding time and there is no significant difference between the bleeding time before and after the application of MSVC extract in different concentration.

Recommendations

1. For ease of delivery to affected sites, it is recommended to develop the extract into a gel product.
2. For future studies, the researchers would like to recommend to include in the methodology the taking of the vital signs of each rat as a modifying factor in the study.
3. Testing for the minimum inhibitory concentration of the extract is also recommended.
4. Larger number of sample is recommended to be tested in the future studies if possible.
5. The antibacterial properties of MSVC peel extract is also suggested to be analyzed. This can increase the scope and properties of the extract for the wound.
6. A toxicity test is suggested to prove the MSVC peel extract’s safety.
7. It is recommended to use 50% concentration in the future studies


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