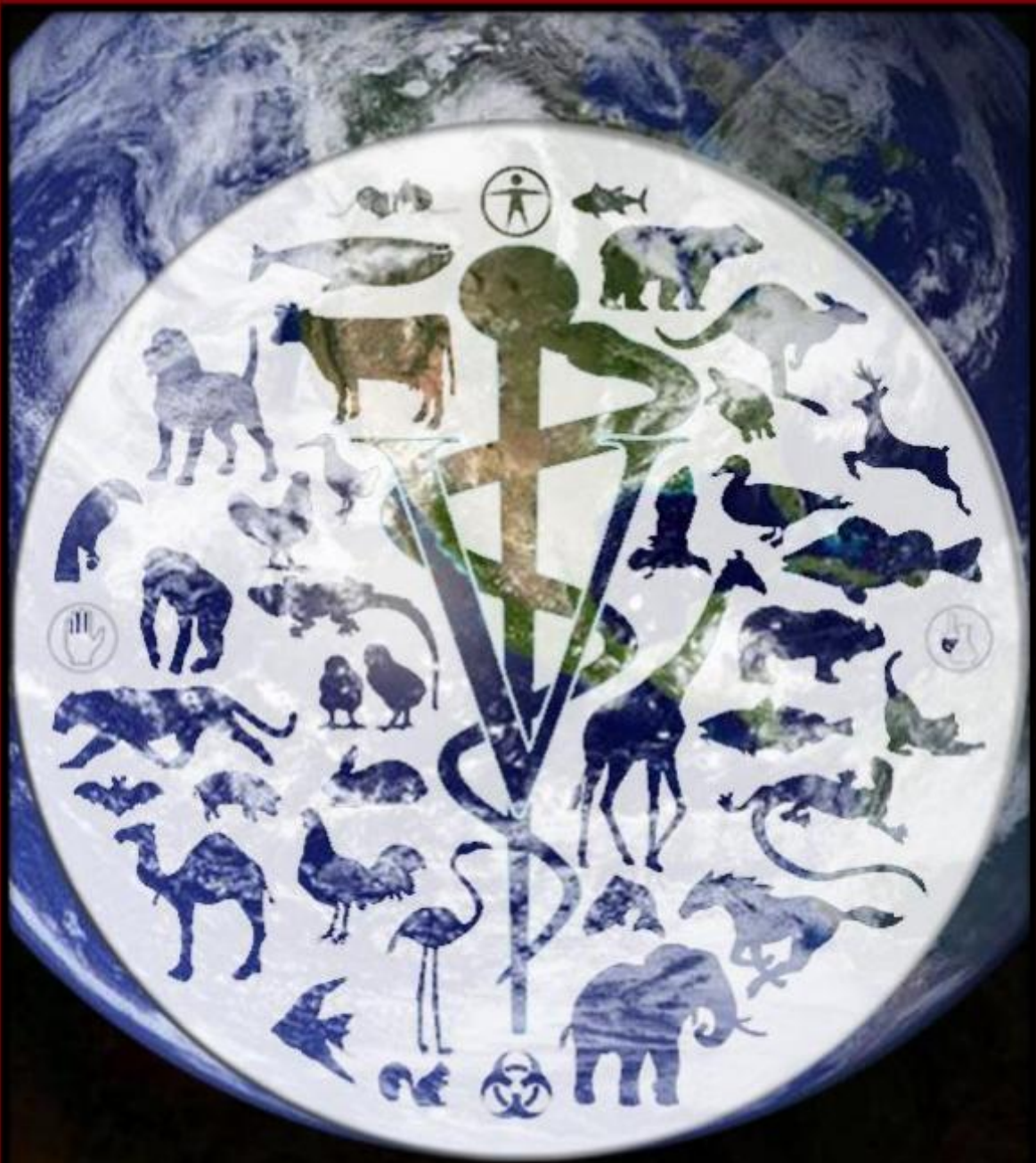


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Review

Antiviral Effects of Plant Extracts Used in the Treatment of Important Animal Viral Diseases

Gamil Zeedan GS and Abdalhamed AM.

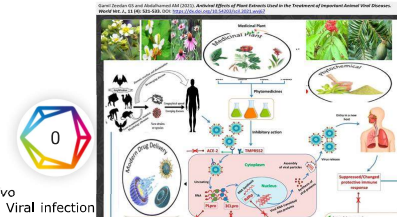
World Vet. J. 11(4): 521-533, 2021; pii:S232245682100067-11

DOI: <https://dx.doi.org/10.54203/scil.2021.wvj67>

ABSTRACT: The goal of this review was to highlight some plant species that have significant antiviral activity against DNA and RNA viruses *in vitro* and *in vivo* although more research is needed to address safety issues, drug interactions, and the possibility of using them in combination with other natural products. Viral infection plays an important role in human and animal diseases. Although there have been advances in immunization and antiviral drugs, there is still a lack of protective vaccines and effective antiviral drugs in human and veterinary medicine. The lack of effective antivirals necessitates the search for new effective antiviral compounds. Plants are naturally gifted at synthesizing antiviral compounds. They are rich sources of phytochemicals with different biological activities, including antiviral activities as a result of advanced analytical chemistry, standard virus assays, and development of standardization and extraction methods. Plant extracts have a wide variety of active compounds, including flavonoids, terpenoids, lignans, sulphides, polyphenolics, coumarins, saponins, furyl compounds, alkaloids, polyenes, thiophenes, proteins, and peptides. Moreover, certain volatile oils have indicated a high level of antiviral activity. Replication, assembly, and release, as well as targeting virus host-specific interactions capable of inhibiting several viruses, could help the development of broad-spectrum antivirals for the prevention and control of viral pathogens. The *in vitro* antiviral activities of *Erythroxylum deciduum*, *Lacistema hasslerianum* (*chodat*), *Xylopiya aromatica*, *Heteropteris aphrodisiaca*, *Acacia nilotica* (*gum arabic tree*), *Lippia graveolens* (*Guettarda angelica* (Velvetseed)), *Prunus myrtifolia*, and *Symphopappus* plant extracts can inhibit viral replication, and interfere with the early stages of viral adsorption of DNA viruses. However, *Boesenbergia rotunda* plant extracts have inhibited RNA viruses. A potent anti-SARS-CoV-2 inhibitor with *B. rotunda* extract and *panduratin A* after viral infection drastically suppresses SARS-CoV-2 infectivity in Vero E6 cells.

Keywords: Bovine herpes virus type-1, Bovine infectious, Bovine infectious cell protein 4, Buffalo pox virus, Foot-and-mouth disease, Plant extracts, Rotaviruses

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Review

Clinical Diagnosis of Leptospirosis in Malaysia: Challenges and Prospects

Garba B and Moussa AA.

World Vet. J. 11(4): 534-542, 2021; pii:S232245682100068-11

DOI: <https://dx.doi.org/10.54203/scil.2021.wvj68>

ABSTRACT: Leptospirosis is a neglected emerging zoonosis occurring both in urban environments and rural regions worldwide. During occupational and recreational activities, individuals who directly or indirectly contact the urine of infected animals are at a high risk of infection. Southeast Asia is reported to have the highest incidence of leptospirosis in the world. The disease is endemic in Malaysia and has consistently caused outbreaks among humans with severe outcomes. Early diagnosis is vital for commence treatment thereby minimizing the harmful effects of the disease. It also allows the implementation of measures to control the spread to humans and the environment. In this article, an attempt was made to review the current diagnostic methods including challenges faced by healthcare service providers during the diagnosis of acute cases, as well as the emerging technologies used for early and accurate diagnosis. The review also highlighted innovative ideas that can be integrated into developing practical solutions, including the crucial roles of the patients, reservoir hosts, and the environments that harbor the bacteria.

Keywords: Emerging zoonoses, Laboratory diagnosis, Leptospirosis, Malaysia, Neglected tropical disease

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Review

Sustainable Rabbit Production under the Global Warming Conditions in Southern Mediterranean Region

El Sabry MI, Zaki MM, Elgohary FA, and Helal M.

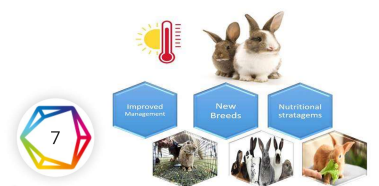
World Vet. J. 11(4): 543-548, 2021; pii:S232245682100069-11

DOI: <https://dx.doi.org/10.54203/scil.2021.wvj69>

ABSTRACT: There is still a wide gap between meat demand and meat production in Egypt. This gap is becoming larger due to the direct and indirect impacts of global warming that negatively influence the grazing area and the performance of livestock. In this context, rabbits are considered as an alternative source for animal-origin meat. In the Southern Mediterranean region, the most obvious problem encountering rabbit production is the hot waves during the summer, especially during the last decades. The narrow thermo-neutral zone of rabbits makes the high temperature hinder the success of commercial production. Different strategies may assist rabbits in coping with heat stress, which are reviewed in the current study, including the improved management practices, development of new breeds, and implementation of different nutritional strategies.

Keywords: Global warming, Rabbit production, Southern Mediterranean

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Research Paper

Global Seroprevalence of West Nile Virus in Horses of Morocco

Hanafi Houiten G, El Berbri I, Mahir W, Aalilouch K, Bouabid B, Zientara S, Alyakine H, El Harrak M, and Fassi Fihri O.

World Vet. J. 11(4): 549-555, 2021; pii:S232245682100070-11

DOI: <https://dx.doi.org/10.54203/scil.2021.wvj70>

ABSTRACT: West Nile Fever (WNV) is a viral emerging mosquito-borne disease causing mortality and morbidity with varying severity (from mild fever to severe neuroinvasive disease) among human and animal populations in many parts of the world. The current study aimed to confirm the virus circulation and assess the disease seroprevalence in horses of Morocco. A sample of 1171 healthy non-vaccinated (against-WNV) horses, taken from 11 (out of 12) regions of the country during July-December 2016, was primarily tested using competitive ELISA assay (cELISA). All cELISA-reactive positive and doubtful sera (n= 269) were further tested by virus neutralization test (VNT). The results of cELISA test revealed an overall WNV seroprevalence in 21.8% (255/1171) of sampled horses. This rate decreased to 18.8% (220/1171) after the confirmation of VNT. The WNV seroprevalence in the current study varied significantly by age, gender, and breed of the tested horses. Indeed, the higher seropositivity rates were found in the oldest (27.7%), female (22.0%), and saddle (32.1%) horses. However, the origin of animals did not show any significant effect on the West Nile virus infection. The obtained results of the present study, therefore, provided serological and epidemiological evidence of the endemicity of the WNV in horse populations of Morocco.

Keywords: Emerging disease, Morocco, Mosquito-borne disease, Seroprevalence, West Nile virus.

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Research Paper

Sensitivity and Specificity of Serology, Histopathology, and Molecular Tests in the Detection of Leptospirosis from Slaughtered Cattle in Indonesia

Rahayu A, Prakoso YA, Desiandura K, and Kristianingrum YP.

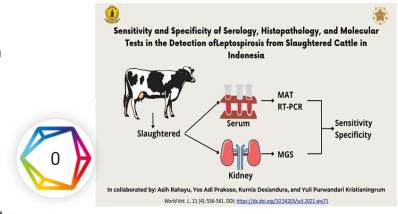
World Vet. J. 11(4): 556-561, 2021; pii:S232245682100071-11

DOI: <https://dx.doi.org/10.54203/scil.2021.wvj71>

ABSTRACT: *Leptospira* spp. is a pathogenic bacteria that causes leptospirosis in humans and cattle. The World Health Organization (WHO) recommends the microscopic agglutination test (MAT) as the laboratory gold standard in the detection of leptospirosis. However, the limitation of MAT triggers the laboratory technicians to develop alternative laboratory tests against leptospirosis. The current study aimed to compare the sensitivity and specificity of histopathology special stain using modified Gram staining (MGS) and molecular test using reverse transcriptase-polymerase chain reaction (RT-PCR), compared to the MAT for *Leptospira* spp. detection in cattle. This study used a total of 38 serum and 38 kidney samples from the cattle slaughtered in the Sidoarjo slaughterhouse, Indonesia. The collected serum samples were tested against MAT and RT-PCR. The kidneys were processed for histopathology using MGS. The result indicated that 16 (42.10%) of the tested samples were positive against MAT, 6 (15.78%) were positive against MGS, and 18 (47.36%) were positive against RT-PCR. The RT-PCR indicated better sensitivity and lower specificity, compared to MAT and MGS. The findings revealed that the RT-PCR is an appropriate laboratory test for detecting cattle leptospirosis with better sensitivity and specificity. Therefore, this method can be suggested to substitute MAT and overcome its limitations.

Keywords: Cattle, Histopathology, *Leptospira* spp., Molecular, Serology

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Research Paper

Impact of Replacing Different Levels of *Panicum maximum* in Rabbit Diets on Growth Performance, Hemato-Biochemical Profile, and Histological Responses of Some Internal Organs

El-Folly IAE-R, Abou Sekken MS, Abo-Eid HE-S, El-Samahy HS, and Mourad DM.

World Vet. J. 11(4): 562-577, 2021; pii:S232245682100072-11

DOI: <https://dx.doi.org/10.54203/scil.2021.wvj72>

ABSTRACT: There has been an interest in alternative sources in rabbit feeding. Therefore, the current study aimed to estimate the health status, growth performance, hemato-biochemical, and histological picture of some important internal organs in growing New Zealand rabbits as a result of replacing different levels of *Panicum maximum* (PM) in its pelleted diet. A total of 35 weaned rabbits (20 males and 15 females) aged 5 weeks were purchased with an average body weight of 839.7 ± 7.05 g and 771.20 ± 9.19 g for males and females, respectively. Randomly, five equal rabbit groups were formed (7 rabbits in each group). The first group (control) was fed a basal diet without PM. The second, third, fourth, and fifth groups were fed pelleted diets containing PM with a replacing percent of 25%, 50%, 75%, and 100% of clover hay, respectively. All groups were fed *ad libitum* of pelleted feed for two months. The blood was aspirated individually three times, including at the beginning of the experiment (as zero time), after one month, and at the end of the experiment, respectively. The whole blood was used for the measurement of hemoglobin concentration, hematocrit percentage, erythrocytes, and total leukocyte counts. At the experimental end, in each group, 3 male rabbits were sacrificed and their internal organs including liver, kidney, cecum, and rectum were collected for histopathology. The live body weight was significantly affected by sex where males were heavier than females, also feed conversion ratio, growth rate, and feed intake were significantly affected by feeding on different levels of PM. Blood hemoglobin, hematocrit, and the total leukocyte count had a non-significant effect while the erythrocyte count increased significantly in all experimental groups. There were insignificant changes in plasma total protein, albumin, globulin, ALT, AST, creatinine, and glucose concentrations when different levels of PM were added. Furthermore, the plasma total cholesterol and triglycerides were significantly decreased in rabbits fed PM, 75% and 100% when compared with 25%, 50%, and control groups. Finally, replacement PM instead of clover hay in pelleted diets till 75% was found to be the safety and optimum percentage for biological and healthy rabbits.

Keywords: Growth, Hemato-biochemical, Histopathology, *Panicum maximum*, Rabbits

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Research Paper

The Effects of Calcination on Mineral Composition and Physical Properties of Limestones and Oyster Shells Derived from Different Sources

Khalil, Rusli RK, and Andri.

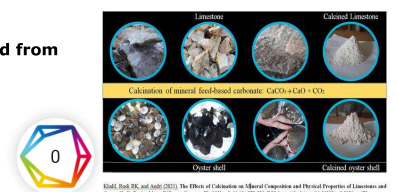
World Vet. J. 11(4): 578-586, 2021; pii:S232245682100073-11

DOI: <https://dx.doi.org/10.54203/scil.2021.wvj73>

ABSTRACT: Limestones and oyster shells are normally used in raw or roasted meal form in the livestock diet. Calcination is intended to improve the mineral concentration and physical characteristics of limestones and oyster shells which vary based on different chemical compositions, textures, and impurities of their types and habitats. The present research aimed to study the effects of calcination on mineral composition and the physical properties of limestones and oyster derived from various sources. Limestone samples from three local limestone mining and oyster shell samples from three shellfish species were calcined by burning at a temperature of 800-1000°C. The calcined products were analyzed for mineral content (Ca, P, Mg, Cu, Zn, and Mn), physical properties (bulk density, tapped density, specific density, and angle of repose), and particle size. Results indicated that calcination had no significant effect on Ca and P concentrations but reduced micro mineral concentration. Limestones had a higher Mg concentration than that of oyster shells, and calcination increased Mg concentration. Calcined oyster shells had higher densities, percentages of fine particles, and lower angles of response. The results suggested that the type of limestones and oyster shells could determine their thermal decomposition properties.

Keywords: Calcination, Limestone, Mineral composition, Oyster shell, Physical property

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Research Paper

Occurrence of Aflatoxin B1 in Animal Feed Collected from the Northeastern Area of Morocco

Alahlah N, El Maoudi M, Bouchriti N, Triqui R, Stitou M, Hafid NH and El Ouahabi O.

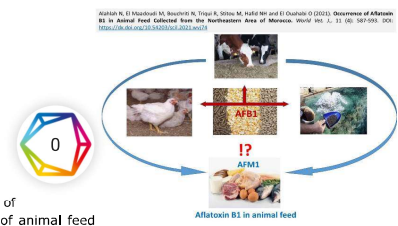
World Vet. J. 11(4): 587-593, 2021; pii:S232245682100074-11

DOI: <https://dx.doi.org/10.54203/scil.2021.wvj74>

ABSTRACT: The carry-over of contaminants from feed to animal products is an important issue in the animal production chain, therefore, the quality control of those animal products should include the control of the animal feed. The current study was carried out to assess the contamination levels of three types of animal feed (dairy animal feed, poultry feed, and fish feed) by Aflatoxin B1. A total of 68 animal feed samples were collected from the Northeastern Moroccan area (Tangier-Tétouan-AL Hoceima). The samples were extracted with a mixture of acetone/water. The sample extractions were filtered, diluted with phosphate-buffered saline, and applied to an immunoaffinity column. Aflatoxin B1 was eluted with methanol then analyzed by high-performance liquid chromatography with fluorescence detection, after post-column photochemical derivatization. The analytical results for the level of Aflatoxin B1 in the animal feed samples revealed an average presence of 44.12% for all analyzed samples. The concentrations were between 1.02 and 13.59 µg/Kg, with a mean value of 4.08 ± 3.11 µg/Kg. The results indicated that there was a significant difference across the three types of animal feeds regarding the concentrations of Aflatoxin B1.

Keywords: Aflatoxin B1, Animal feed, HPLC, Morocco, Statistical data

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Research Paper

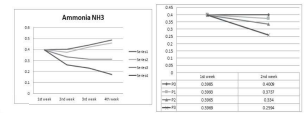
Research Paper

Potential Carbon Thickness on Ammonia Content in Nile Tilapia (*Oreochromis niloticus*) Aquaponics System with Water Spinach (*Ipomoea aquatica*)

Abizaka FT, Mahasri G, and Nindarwi DD.

World Vet. J. 11(4): 678-684, 2021; pii:S232245682100085-11

DOI: <https://dx.doi.org/10.54203/scil.2021.wvj85>



Potential Carbon Thickness on Ammonia Content in Nile Tilapia (*Oreochromis niloticus*) Aquaponics System with Water Spinach (*Ipomoea aquatica*)

ABSTRACT: The application of aquaponics aquaculture is needed to improve water quality, especially the addition of filtration materials which act as absorbent planting mediums for ammonia nitrogen content in toxic water. The purpose of the current study was to determine the effect of the carbon thickness on ammonia levels in Nile tilapia in aquaponics systems with Water spinach. The study used an experimental method with a completely randomized design consisting of four treatments and five replications. The main parameter was ammonia level. Supporting parameters in this study included initial and final growth of Water spinach, fish survival rate, specific growth rate, and water quality which included temperature measurement, dissolved oxygen, and pH. Each treatment consisted of variations of carbon thicknesses at P0 (control), P1 (5 cm), P2 (7 cm), and P3 (9 cm). The results revealed that ammonia levels were significantly different at P1, P2, and P3 in the third and fourth weeks. Ammonia levels during the study decreased from 0.3969 ppm to 0.1741 ppm. The reported value of 7.2 was for acidity degree, 29.3°C for the temperature, 5.94 ppm for dissolved oxygen, 8.42 cm for the growth of Water spinach, 0.44% for the specific growth rate, and 90% for the survival rate. Carbon thickness caused a decrease in ammonia levels in Nile tilapia with aquaponics systems through the medium of Water spinach.

Keywords: Ammonia, Carbon thickness, *Ipomoea aquatica*, *Oreochromis niloticus*

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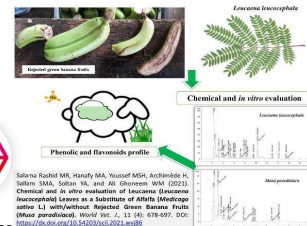
Research Paper

Chemical and *in vitro* evaluation of *Leucaena leucocephala* Leaves as a Substitute of Alfalfa (*Medicago sativa* L.) with/without Rejected Green Banana Fruits (*Musa paradisiaca*)

Salama Rashid MR, Hanafy MA, Youssef MSH, Archimède H, Sallam SMA, Soltan YA, and Ali Ghoneem WM.

World Vet. J. 11(4): 685-697, 2021; pii:S232245682100086-11

DOI: <https://dx.doi.org/10.54203/scil.2021.wvj86>



Chemical and *in vitro* evaluation of *Leucaena leucocephala* Leaves as a Substitute of Alfalfa (*Medicago sativa* L.) with/without Rejected Green Banana Fruits (*Musa paradisiaca*)

ABSTRACT: *Leucaena* leaves and rejected green banana fruits can be promising to cope with feed gaps in arid and semi-arid Mediterranean regions. The present study evaluated the feeding value and secondary active compounds of *Leucaena* leaves and rejected green banana fruits for ruminants using a semi-automated gas production (GP) system. Comparisons were made with the traditional feeds as alfalfa, and *Dichanthium* spp. grass hay. Analysis of HPLC was performed for the feed ingredients to characterize the main phenolic components. The *in vitro* evaluation was carried out for the experimental feed ingredients and diets. Four diets were formulated as the first diet consisted of alfalfa and grass hay at a ratio of 35:65 (AG), the second diet composed of alfalfa, grass hay, and green banana fruits at a ratio of 35:55:10 (AGB), third and fourth diets were prepared by replacing alfalfa in AG and AGB with *Leucaena* leaves to be LG and LGB, respectively. *Leucaena* leaves showed a high content of valuable phenolic components that have antioxidant and anti-inflammatory properties, such as gallic acid, ellagic acid, and naringenin. Moreover, *Leucaena* leaves and diet had higher crude protein, total phenols, and total tannins than alfalfa, which was reflected on the chemical composition of diets, and recorded the lowest total accumulative GP at 24 hours leading to low CH_4 and CO_2 production. Banana fruits recorded the lowest ruminal pH, ammonia concentration, and degraded neutral detergent fiber, compared to other feed ingredients, while it had the highest GP and degraded organic matter. Therefore, it is highly recommended to use *Leucaena* leaves in animals' diets with/without rejected green banana fruits as an alternative feed resource with potential environmental and animal health benefits.

Keywords: Alfalfa, *Dichanthium* spp. grass, Green banana fruits, *In vitro* evaluation, *Leucaena leucocephala* leaves, Phenolic compounds

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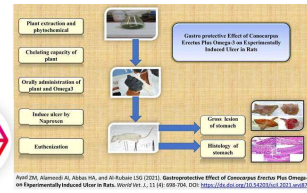
Research Paper

Gastroprotective Effect of *Conocarpus Erectus* Plus Omega-3 on Experimentally Induced Ulcer in Rats

Ayad ZM, Alameedi AI, Abbas HA, and Al-Rubaie LSQ.

World Vet. J. 11(4): 698-704, 2021; pii:S232245682100087-11

DOI: <https://dx.doi.org/10.54203/scil.2021.wvj87>



Gastroprotective Effect of *Conocarpus Erectus* Plus Omega-3 on Experimentally Induced Ulcer in Rats

ABSTRACT: There has been a dearth of research on the gastroprotective effect of *Conocarpus erectus* in the literature so the current study was designed to estimate the ability of *Conocarpus erectus* (*C. erectus*) leaves extract alone and in combination with omega-3 regarding gastroprotective effects. A total of 30 male rats were divided into five groups (n = 6). All animals induced gastric ulcer by 80 mg/kg of naproxen orally twice a day for three consecutive days. At the same time, the animals treated orally with 175 mg/kg omega-3, 250 mg/kg *C. erectus*, 80 mg/kg omega-3 + 150 mg *C. erectus*, 10 mg/kg of lansoprazole, and 2 ml/kg of DMSO were named T1, T2, T3, T4, and TC, respectively. The obtained results of the present study indicated the presence of flavonoids, saponin, and tannin as active ingredients in *C. erectus* leaves extract. Consequently, *C. erectus* seemed to have the potential of chelating metals in a concentration-dependent manner. Gross and histopathology findings showed the highly protective capability of *C. erectus* and omega-3 against ulcerative lesion, compared to the time each was used alone. The outcomes of the current study indicated that using *C. erectus* alone or plus omega-3 can protect the gastric mucosa from the ulceration induced by naproxen, and the chelating properties of *C. erectus*.

Keywords: *Conocarpus erectus*, Naproxen, Omega-3, Rat, Ulcer

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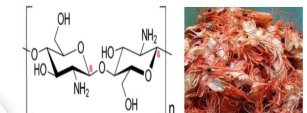
Short Communication

Potential of Chitosan from Shrimp Waste for Treating *Staphylococcus aureus* Bacteria in Skin Wound

Widnyana IMS, Subekti S, and Kismiyati.

World Vet. J. 11(4): 705-708, 2021; pii:S232245682100088-11

DOI: <https://dx.doi.org/10.54203/scil.2021.wvj88>



Potential of Chitosan from Shrimp Waste as a Skin Ointment for Treating *Staphylococcus aureus* Bacteria in Skin Wound

ABSTRACT: Shrimp or crustacean waste on the skin, head, and feet has not been maximally utilized. Shrimp or crustacean waste can be processed into chitin or chitosan which can provide high added value. Chitosan is one ingredient that can be used to make skin ointments. One of the bacteria that is harmful to the skin during a wound is *Staphylococcus aureus*. Hence, there is a need to have a skin ointment that can inhibit these bacteria. The current study aimed to evaluate the antibacterial activity of chitosan in the ointment. Moreover, the current study planned to determine the optimum inhibitory concentration of chitosan in the ointment against *Staphylococcus aureus* and evaluate the physical properties of ointments. The methods used in the current study were the pH determination, organoleptic test, homogeneity test, and antibacterial activity test to examine chitosan against *Staphylococcus aureus*. The results showed that chitosan in ointment had a weak antibacterial activity with a value of < 5 mm inhibition zone. Chitosan with a concentration of 0.2% in ointment fulfilled the requirements of a good ointment. Its white colour as the typical colour of chitosan showed the characteristic odour of chitosan and a semi-solid form. Chitosan in the ointment has antibacterial activity that can inhibit *Staphylococcus aureus* bacteria.

Keywords: Chitosan, Skin Ointment, *Staphylococcus aureus*.

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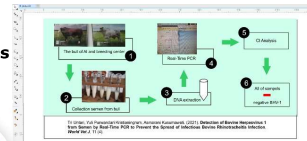
Short Communication

Detection of Bovine Herpesvirus 1 from Semen by Real-time PCR to Prevent the Spread of Infectious Bovine Rhinotracheitis Infection

Untari T, Kristianingrum YP, and Kusumawati A.

World Vet. J. 11(4): 709-712, 2021; pii:S232245682100089-11

DOI: <https://dx.doi.org/10.54203/scil.2021.wvj89>



Detection of Bovine Herpesvirus 1 from Semen by Real-time PCR to Prevent the Spread of Infectious Bovine Rhinotracheitis Infection

ABSTRACT: Infectious Bovine Rhinotracheitis (IBR) can be transmitted by livestock seeds and semen, through the process of artificial insemination. Therefore, it is necessary to detect the presence of the Bovine Herpesvirus 1 (BHV1) in semen through artificial insemination and breeding centers of Indonesia. The current study aimed to detect the presence of the virus in semen as a source of IBR disease transmission in Indonesia. A total of 27 semen samples from artificial insemination and breeding centers (Sembawa, Lembang, Ungaran, and Sleman) in Indonesia have been examined and identified using the real-time PCR (qPCR) technique. The result showed that all samples were negative to BHV1. This indicated that semen from Ungaran, Sembawa, Sleman, and Lembang was safe to be used as a semen source for artificial insemination.

Keywords: Artificial insemination, Bovine Herpesvirus 1, Infectious Bovine Rhinotracheitis, Real-time PCR, Semen

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Research Paper



Potential of Chitosan from Shrimp Waste for Treating *Staphylococcus aureus* Bacteria in Skin Wound

I Made Satya Widnyana¹, Sri Subekti^{1*}, and Kismiyati²

¹Department of Marine Science, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya, East Java, Indonesia

²Department of Fish Health Management and Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya, East Java, Indonesia

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ABSTRACT

Shrimp or crustacean waste on the skin, head, and feet has not been maximally utilized. Shrimp or crustacean waste can be processed into chitin or chitosan which can provide high added value. Chitosan is one ingredient that can be used to make skin ointments. One of the bacteria that is harmful to the skin during a wound is *Staphylococcus aureus*. Hence, there is a need to have a skin ointment that can inhibit these bacteria. The current study aimed to evaluate the antibacterial activity of chitosan in the ointment. Moreover, the current study planned to determine the optimum inhibitory concentration of chitosan in the ointment against *Staphylococcus aureus* and evaluate the physical properties of ointments. The methods used in the current study were the pH determination, organoleptic test, homogeneity test, and antibacterial activity test to examine chitosan against *Staphylococcus aureus*. The results showed that chitosan in ointment had a weak antibacterial activity with a value of < 5 mm inhibition zone. Chitosan with a concentration of 0.2% in ointment fulfilled the requirements of a good ointment. Its white colour as the typical colour of chitosan showed the characteristic odour of chitosan and a semi-solid form. Chitosan in the ointment has antibacterial activity that can inhibit *Staphylococcus aureus* bacteria.

Keywords: Chitosan, Skin Ointment, *Staphylococcus aureus*.

INTRODUCTION

Regarding the antibacterial, antifungal, and antiviral activities of chitosan, it is necessary to develop a product in the pharmaceutical field to benefit from such properties (Chouhan et al., 2017). One of the product preparations in the pharmaceutical field that can facilitate its use is ointment (Ibezim et al., 2018).

Therefore, the current study was conducted to determine the inhibitory concentration of chitosan in ointment against *Staphylococcus aureus* and evaluate the physical properties of skin ointment preparations.

MATERIALS AND METHODS

Variables

The independent variables of the current study included variations in the concentration of chitosan in the ointment. The control variable was chitosan in the ointment, and the heating temperature of chitosan in the ointment was 70°C, while the dependent variable was inhibition of *Staphylococcus aureus* and physical phlegm ointment.

Study design

This study used four treatments in testing the physical properties of ointment preparations, namely K as the control (commercial ointment), P1 (0.05% chitosan ointment), P2 (0.1% chitosan ointment), and P3 (0.2% chitosan ointment).

Procedures

The percentage of chitosan concentration in ointments in the current study was based on the study of Chiba et al. (2006) as the amounts of chitosan investigated for topical doses ranged 0.1-5%. Chitosan at smaller amounts can improve skin wound healing. The analysis of the chitosan-based ointment in the current study started with investigating the physical properties of ointment. In this regard, the pH test (degree of similarity), organoleptic test, and homogeneity test were conducted. The second phase of the study involved the *Staphylococcus aureus* inhibitory test according to Yasrebi et al. (2020).

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Statistical analysis

To analyze the obtained results, one-way ANOVA test was used followed by Duncan in SPSS version 22. The level of significance was set at $p < 0.05$.

RESULTS

Chitosan pH test in the ointment

The pH meter was used to determine the pH of the chitosan-based ointment. The obtained results indicated that chitosan-based ointments had the lowest and the highest average pH of 5.87 and 6.15, respectively. The ideal pH for the skin ointment was 4.5-6.5. Chitosan with concentrations of 0.05%, 0.1% and 0.2% in ointments are good for the skin. One-way ANOVA test results on the pH test of chitosan in the ointment showed that there were significant differences in pH values between various treatments ($p < 0.05$). The pH test of chitosan in ointment had a significant effect on microbial inhibition in the current study ($p < 0.05$).

Inhibitory tests

One-way ANOVA test results on the pH test of chitosan in the ointment showed that there were significant differences in pH values between various treatments ($p < 0.05$). The pH test of chitosan in ointment had a significant effect on microbial inhibition in the current study. The inhibitory test of chitosan bacteria in ointment was conducted to determine the ability of ointments to inhibit the growth of *Staphylococcus aureus* bacteria and to determine an effective and efficient treatment system. Observations of bacterial inhibition tests were conducted by calculating the diameter of the clear zone and the diameter of What-mann paper. Antibacterial commercial ointment inhibitory zone activity had an inhibition zone of 3.4 mm, ointment with chitosan concentrations of 0.05%, 0.1%, and 0.2% had an inhibition zone of 1.3 mm, 1.6 mm, and 4 mm, respectively. The commercial ointment and chitosan-based in ointments had weak antibacterial activity with a value of 5 mm. The inhibition of bacteria as < 5 indicates the weak, 5-10 mm shows moderate, 11-20 reveals strong, and > 20 signifies the very strong activity. One way-ANOVA test results on the antibacterial activity of chitosan in the ointment showed no significant difference among the treatments in terms of their bacterial inhibition zone ($p > 0.05$).

In the current study, the inhibitory test of chitosan bacteria in ointment did not significantly influence the inhibition zone of the bacteria. There was a weak category of antibacterial activity in chitosan of the ointment with a value of ≤ 5 mm. Chitosan in ointment with a concentration of 0.2% has an inhibition zone diameter that was greater than the control treatment which only consists of an ointment base. This was because chitosan has antibacterial properties that can kill bacteria. The greater the concentration of chitosan in the ointment, the greater the inhibitory zone formed.

Homogeneity test

The observations made on the homogeneity of the ointment base and chitosan in the ointment showed homogeneous, identical, and no coarse particles in the ointment. The chitosan homogeneity test results in ointments of 0.05%, 0.1%, and 0.2% have the same physical properties.

Organoleptic test

In organoleptic testing, the colour, shape, and odour of the commercial ointment and chitosan-based ointment were evaluated. The first was the test of colour which evaluated the base of the ointment and the chitosan in the ointment in terms of colour. The colour was a yellowish white colour for chitosan-based ointment concentrations of 0.05% and 0.1% as well as the commercial ointment. However, 0.2% of chitosan in the ointment led to the formation of white colour. The Organoleptic Test for odour was used to observe the commercial ointment and chitosan in the ointment regarding aroma. The obtained results indicated that commercial ointment had a distinctive odour of the ointment. While the observation of ointment with 0.05% chitosan indicated a distinctive odour of ointment, 0.1% and 0.2% concentration of chitosan in the ointment led to a distinctive odour of shrimp. The Organoleptic Test of shape was employed for chitosan with concentrations of 0.05%, 0.1%, and 0.2% in ointments in terms of shape and physical form. Kruskal Wallis test results of organoleptic tests on colour, odour, and shape revealed significant differences among the treatments ($p < 0.05$).

DISCUSSION

The results obtained at the concentration of 0.2% chitosan in ointment had the largest inhibitory zone diameter, compared to the control treatment (ointment base), chitosan concentration of 0.05% in the ointment, and chitosan concentration of 0.1% in the ointment. It can be stated that the greater the diameter of the inhibition zone formed or the higher the concentration of chitosan in the ointment, the greater is effect on inhibiting the growth of *Staphylococcus aureus*.

The PH testing in the current study was very important because it is in direct contact with the skin thus it would affect the condition of the skin (Schmid-Wendtner and Korting, 2006). The ointment pH test was conducted to determine the acidity and basicity of the ointment preparation to the skin. A good pH value of ointment is 4.5, - 6.5 or by the pH value of human skin (Lamberts et al., 2006).

The smaller the pH or the more acidic the preparation, the easier it is to irritate the skin while the higher the pH value can make the skin dry, therefore, pH testing was very important in making topical preparations thus preparations made do not irritate the skin when used (Lamberts et al., 2006). The chitosan with concentrations of 0.05%, 0.1%, and 0.2% in ointment did not irritate if applied to the skin so it is a good ointment requirement. One-way-ANOVA test results on the chitosan pH test in ointments showed that there were significant differences between various treatments ($p < 0.05$). The PH test of chitosan in ointment had a significant effect in this study.

Homogeneity testing in the current study was conducted to find out if the ointment preparations was evenly mixed. The homogeneous ointment was characterized by the absence of lumps in the basting result until the end of basting (Barkah, 2016). Homogeneity test results performed on each ointment proved homogeneity if there were no clumping particles and had the same colour on all parts of the ointment. The homogeneity of the ointment can also affect the dose of the ointment when it was used, a homogeneous ointment will give an even dose (Carneiro and Poppi, 2012).

Homogeneous ointment preparations would provide good results because the medicinal ingredients are evenly dispersed in the basic ingredients, hence in each part, the preparations contain the same amount of medicinal ingredients (Abuyeva et al., 2018). If the ingredients of the drug were not dispersed evenly in the basic ingredients, then the drug will not achieve the desired therapeutic effect (Abuyeva et al., 2018). The observations made on the homogeneity of the ointment base and chitosan in the ointment showed homogeneity, evenness, no coarse grains, and lumps in the ointment. The chitosan homogeneity test results in ointments (concentrations of 0.05%, 0.1%, and 0.2%) have the same physical properties meaning that homogeneity was observed in various treatments and each repetition.

Organoleptic testing in the current study was conducted by observing the ointment preparations from the shape, odour, and colour of the preparations (Barkah, 2016). In organoleptic testing, the colour, shape, and odour of the commercial ointment and chitosan-based ointment were with concentrations of 0.05%, 0.1%, and 0.2% were compared. Observation of the commercial ointment and chitosan-based ointment showed the colour difference of each concentration. The colour formed was yellowish-white in the observation of the ointment base, this is due to the mixing of the white Vaseline albumins and the lanæ adeps and cera alba which were yellow which is the typical colour of the mixture of the two ointment bases.

Observation of chitosan in ointment concentration of 0.05% colour that was formed was yellowish-white colour, chitosan in ointment concentration of 0.1% colour formed was yellowish-white colour, and chitosan in ointment concentration of 0.2% colour that was formed was white. This was because the more the concentration of the solution based on the ointment, the colour of the ointment will be more similar to the colour characteristics of the solution used using white chitosan shrimp in this study. Chitosan in ointment concentration of 0.2% was the best ointment due to the organoleptic test results, the colour formed was white which was the typical colour of chitosan (shrimp). The colour of a good ointment was the distinctive colour or carrier of the ointment preparation (El-Gied et al., 2015).

Observation of the ointment base and chitosan in the ointment regarding odour showed the ointment base has a characteristic ointment odour. This is because the ointment base material used was the ointment produced from oil purification. However, the observation of chitosan in ointment concentration of 0.05% has a distinctive odour of ointment, chitosan in ointment with the concentration of 0.1% has a distinctive odour of chitosan (shrimp), and chitosan in the ointment of concentration of 0.2% has a characteristic odour of chitosan (shrimp). This is because the solution contained mask the odour from the base of the ointment.

Chitosan in ointment concentrations of 0.1% and 0.2% was the best ointment because the results of the organoleptic test showed the odour typical of chitosan (shrimp). The higher the concentration of the solution used in the ointment base, the easier it is to find out the characteristic odour used on the ointment. A good odour was the characteristic odour or carrier of the ointment preparation (El-Gied et al., 2015).

Observations on the basis of ointments and chitosan in ointments concentrations of 0.05%, 0.1%, and 0.2% in terms of shape have the same physical form that was semi-solid. The semi-solid form was a characteristic of ointments. Chitosan in ointment concentrations of 0.05%, 0.1%, and 0.2% was the best ointment because organoleptic test results showed the form of semi-solid form. The form of a good ointment preparation has a physical form that was semi-solid (Bharat et al., 2011).

CONCLUSION

Chitosan in ointment material with a concentration of 0.2% fulfilled the requirements of a good ointment. The white colour which was the typical colour of chitosan showed the characteristic odour of chitosan and a semi-solid form. Chitosan in ointment had an antibacterial activity that could inhibit *Staphylococcus aureus* bacteria.

DECLARATIONS

Competing interests

Authors declare no competing interests.

Authors' contribution

The authors have participated in this study equally.

Ethical consideration

Ethical issues (Including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by the authors.

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