



Investigation Antibacterial Activity Extraction from Two Medicinal Plants Available in Sudan

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Abstract

An aim of present study is investigated the antibacterial activity of petroleum ether and methanol extracts of two Sudanese medicinal plants *Vigna coerulea* Bak. and *Aloe vera* were tested against three species of bacteria, *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus*. Results were revealed the methanol extracts of two medicinal plants were effective on bacterial strains, while the crude plants were weakly effective. The diameter of inhibition zones ranged from 15-20 mm. A minimum inhibitory concentration (MIC) of the plants against different microorganisms was done by using a microdilution method, it was found to be ranged from 0.156 -0.313 mg/ml. In vivo-sensitivity of the plants were tested on 10, three days old by using the white rats. The rats were infected with different bacterial strains. Stained films from the lesions, cultures and histopathology confirmed the infections. The infected wounds showed dramatic response to the usage of the plants as topical treatment. Toxicity of the plants was tested in rats. The plants mixed with distilled water and given to the rats as drinking water. The hematological and chemical parameters were measured before and after ingestion of the plants by rats. Histopathology sections of the hearts, livers, lungs, kidneys and spleens of the rat become normal.

Keywords: Antibacterial, haematology, histopathology and toxicity.

Introduction

In recent years, the multiple drug resistance has developed due to the indiscriminate use of available antimicrobial drugs in treatment of infection diseases. In addition, antibiotics are associated with adverse effects on the host. Therefore, developing alternative antimicrobial drugs from other sources (Medicinal Plants) is required. The medicinal plants are used in the entire world for the treatment against the various infections. Medicinal value of these plants was recognized since ancient times¹ *Vigna coerulea* Baker, locally is known as Ibreeg El-faki, is one of the most widely used ingredients alternative medicine for the treatment of wounds². Aromatic and medicinal plants are known to produce certain bioactive molecules which react with other organisms in the environment inhibiting bacterial or fungal growth^{3,4}. About 30% of drugs used worldwide are based on natural products⁵.

The objectives of this study were investigated in-vitro, in-vivo-sensitivity and toxicity of these plants.

Material and Methods

Collection and preparation of extraction plants: Fresh plants materials were collected from Horticulture Department, University of Alzaiem Al azhari, Sudan. Their botanical identities of the two medicinal plants were determined as shown in figure 1 and 2. The preparations of plants sample were done⁶.

Extractions of the plants: 100gm powder plants from each sample were extracted with 400ml petroleum ether (60-80°C) by using Soxhlet apparatus and then material was successively extracted by 80% methanol and maceration at room temperature. The extracts were filtered by filter paper (Whitman No.1) by inducing pump suction for acceleration the filtrations. Extracts were concentrated to dryness by using the rotatory vacuum and weighed⁷.

In-vitro-sensitivity tests: The antimicrobial activity was done in Muller Hinton media for different bacterial organisms by using agar well diffusion method (cup-plate method)^{8,9}. By means of Cork Borer (8 mm in diameter) a cup is made in a seeded agar plate. The agar disc is removed with a sterile loop and the cup was filled with the plants (crude and extracts) at concentration of 5mg/cup which diffuses from the cup to a certain extent. The plates were incubated at 37°C for 24 hours for bacterial activity. The inhibition zones are measured and recorded comparing with zone of inhibition of the tested organisms with a data base of known sensitivity organisms^{10,11}. Average of two replicates of inhibition zone was evaluated.

Determination of minimum inhibitory concentration (MIC): MIC of the extracts was determined by the microdilution method^{7,10}. The extracts of *Vigna coerulea* Bak and *Aloe vera* were diluted in Mueller Hinton broth to give final concentrations of 10.0, 5.0, 2.5, 1.25, 0.625, 0.313, 0.156,

0.078, 0.039 and 0.0195 mg/ml respectively. The microorganism's inoculum size was adjusted the turbidity of 0.5 Mc Far, so as to deliver final inoculum of approximately 10^5 colony forming unit (CFU/ml). Using a micropipette, 50 μ l of the adjusted microbial broth culture were introduced into the wells. The test plates were incubated at 37C° in 18-24 hours for bacterial activity. The end point (MIC) was recorded as the low concentration of extracts in which the microorganisms tested did not show visible growth⁶.

In-vivo-sensitivity test in animals: Three days old 10 white rats were used for in-vivo-sensitivity study using the crude and extracts of the herbs. The mean weight of the rats was 100 gm. Control rats do not treated with crude and extracts of the herbs. The rats were inoculated with *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis* in wounds body of the rats. The sites of inoculation were examined daily to look for the development of infections. Various specimens were taken to confirm the infections. The specimens collected were wound swab, tissue smear, grains and tissue sections. Microscopic examination was used for gram stain, culture on blood agar and MacConkey was used to confirm the type of the organism causing the infections. After establishing of infections the crude and the extracted herbs were applied topically to the lesions (in forms of ointments with Vaseline). The rats were followed up daily to look for the progress of the lesions figure 3 and 4.

Toxicity of the plants: Four groups of rats were used for toxicity testing. Each group with 8 rats. The weight of each rat ranged from 100 to 150 gm, group C₀ used as a control. Group C₁, C₂ and C₃ were given orally extracts of the two plants dose of 0.5 mg /gm weight, 1 mg/ gm weight and 1.5 mg /gm weight respectively. The extracts of the plants were mixed with distilled water and given to the rats as drinking water.

Daily observations were done to make sure that the mice actually drank the water. This was done for three weeks under strict observations, looking for any clinical signs. Blood samples were collected from the eye initially, at ten days and three weeks for the analysis of different parameters.

Hematological studies: Hemoglobin concentration (Hb), red blood cells counts (R.B.Cs.count), white blood cells count (W.B.C.), mean corpuscular volume (M.C.V.), packed cell volume (P.C.V.) and mean corpuscular hemoglobin concentration (M.C.H.C.) were estimated.

Chemical studies: Serum samples were analyzed for the level of glucose, alkaline phosphatase (A.L.P.), Creatinine, urea, total protein, cholesterol, bilirubin, albumin, aspartate transaminase (AST) and alanine transaminase (ALT).

Histopathology studies: After three weeks the rats were sacrificed. Tissue sections were done for the hearts, livers, spleens, lungs and kidneys for histopathology study. The tissues were fixed in 10% formaldehyde and blocked in paraffin wax.

6 μ m thick sections were stained with haematoxylin and eosin (H&E).



Figure-1
Vigna coerulea Baker (Ibreeg El-faki)



Figure-2
Aloe vera



Figure-3
Control rat with a lesion infected by *Staphylococcus aureus* (day 0)

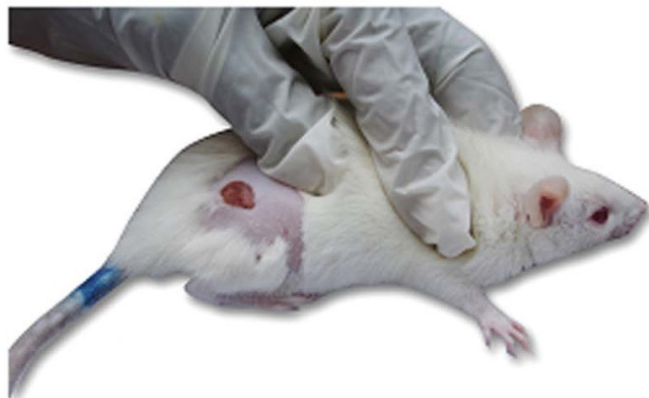


Figure-4

The effect of *Aloe vera* ointment on the lesion infected by *Staphylococcus aureus* (day 2)

Results and Discussion

In-vitro-sensitivity tests of the plants: The antibacterial activity of *Vigna coerulea* Bak. and *Aloe vera* was tested against three bacterium species, *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus*, methanol extracts of these plants were effected on bacterial strains, the crude extracts of these plants were weakly effected. The crude extract of *Aloe vera* was observed effective against *Escherichia coli*, because inhibition zone was 15 mm in diameter. The crude extract of *Vigna coerulea* Bak has no effected against any of the tested organisms. The petroleum ether extract of *Vigna coerulea* Bak. and *Aloe vera* did not show any activities against any of the tested organisms. Methanol extract of *Vigna coerulea* Bak was shown active against *Escherichia coli* because inhibition zone was 15 mm in diameter figure 5, and also on *Staphylococcus aureus* because inhibition zone was 18 mm in diameter figure 6, and inhibition zone of *Bacillus subtilis* was 20 mm in diameter figure 7. The methanol extract of *Aloe vera* was found active against *Staphylococcus aureus* because inhibition zone was 17 mm in diameter, *Bacillus subtilis* (the diameter the inhibition zone was 17mm) and inhibition zone of *Escherichia coli*. was 15mm in diameter. The large inhibition zone (20 mm in diameter) was observed in the methanol extract of *Vigna coerulea* Bak. and on the growth of *Bacillus subtilis*.

The minimum inhibitory concentration of the plants (MIC): The MIC of *Vigna coerulea* Bak. extracts was 0.156 mg/ml against *Bacillus subtilis*, 0.625mg/ml against *Escherichia coli* and 0.313 mg/ml against *Staphylococcus aureus*. The MIC of *Aloe vera* extracts was 0.156 mg/ml against *Bacillus subtilis*, 0.313 mg/ml against *Staphylococcus aureus* and *Escherichia coli*.

In-vivo-sensitivity tests in animals: 10 white rats were used for this test. The growth of the organism was clearly observed in all inoculated rats. Stained films from the lesions, cultures and histopathology was confirmed the infections by bacteria. After usage of the plants as topical treatment for one week to two

weeks, the lesions and wounds were healed dramatically. Control groups were used to prove that healing was not spontaneously.

Toxicity studies: 32 rats were used to find out if there is any toxicity of the plants. The extracts of the plants were mixed with distilled water and given to the rats as drinking water Daily observation was done to make sure that the rats actually drank the water. This was done for three weeks under strict observations. The hematological and chemical parameters, which analyzed before and after ingestion of the plants, were within normal levels. Macroscopically findings revealed that there were no significant macroscopic signs in all groups of animals which were given orally the extracts of the two plants with various doses.

Histopathology of sections from the hearts, livers, lungs, spleens and kidneys of the rats were showed significantly no clinical signs or lesions of rats which were given orally the extracts of the two plants in different doses.

Discussion: In recent years, different reports from different countries were indicated that there were antimicrobial activities of medicinal plants¹¹. The results of the present study indicated an existence antibacterial activity in crude and methanol extracts of *Vigna coerulea* Bak and *Aloe vera*. In-vitro-sensitivity of crude and petroleum ether extracts of *Vigna coerulea* Bak showed that there is no antimicrobial activity against the tested organisms, while the methanol extract of *Vigna coerulea* Bak showed antimicrobial activity against *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli*. In-vitro-sensitivity of crude and extract of petroleum ether of *Aloe vera* showed that there is effective against *Escherichia coli* only. The petroleum ether extract of *Aloe vera* was found to be inactive against the tested organisms, while the methanol extract of *Aloe vera* was active against *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis*. In-vivo-sensitivity of the plants studied on the infected rats proved to be very active. All the infected rats were cured by local application of the plants on the lesions. No spontaneous improvement was detected on the infected control rats. This indicates that the cure of the tested rats was due to the action of these plants studied. Toxicity tests of the plants on rats showed no symptoms or signs of toxicity. This is confirmed by testing the different biochemical parameters as well as examining tissue sections from the essential organs, all the results were the same before and after usages of the plants. These plants are cheap, easy to grow, very effective on the in-vitro and in-vivo, non toxic (safe) easy to apply and can be tested at any time looking for any developing resistance.

Conclusion

In this study, the crude, petroleum ether and methanol extracts of *Vigna coerulea* Bak and *Aloe vera* were found to be active against most bacterial strains. The methanol extracts were more active than crude plants, while the petroleum ether extracts were

weakly effective. The use of those plants in the form of topical therapy in infected rats was proved the affectivity of these *Vigna coerulea* Bak and Aloe vera plants as medicinal purpose. Toxicity tests was showed no clinical significances. The results are support the folkloric usage of *Vigna coerulea* Bak and Aloe vera and suggest that these plants extraction possess compounds with antimicrobial properties that can be further explored for antimicrobial activity.

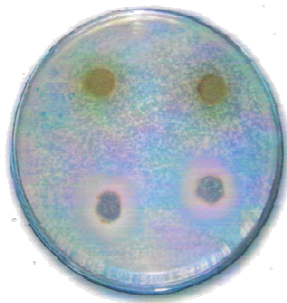


Figure-5

The effect of methanol extract of *Vigna coerulea* Baker on *Escherichia coli*



Figure-6

The effect of methanol extract of *Aloe vera* on *Staphylococcus aureus*



Figure-7

The effect of methanol extract of *Aloe vera* on *Bacillus subtilis*

Abbreviations: Hb = Hemoglobin R.B.Cs = Red blood cells count, W.B.C.= white blood cells count, M.C.V.) = Mean corpuscular volume, P.C.V.= Packed cell volume and M.C.H.C.= Mean corpuscular hemoglobin concentration.

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