Antibacterial properties of compounds from s. frutescens

- Kabir Prema

Introduction

Rationale

There are approximately 6. 1 million people living with the Human Immunodeficiency Virus and Acquired Immune Deficiency Syndrome in South Africa (Unaids. org, 2014). People with HIV/Aids have a higher risk of getting secondary infections and diseases such as Tuberculosis, which is the cause of many deaths in South Africa (Tbfacts. org, 2014). About 5. 5 million people in South Africa are infected with Tuberculosis (Salim S. Abdool Karim, 2009). I have chosen to research and experiment on the Sutherlandia frutescens because it has anti-bacterial and anti-HIV properties (Katerere and Eloff, 2014). I also have a keen interest in alternative medicines so researching and testing a plant with many diverse properties such as *S. frutescens* will be an interesting and fruitful experience for me.

Hypothesis

Compounds extracted from *S. frutescens* have antibacterial properties.

Aim:

To test three extraction methods (water, ethanol and acetone) on *S. frutescens* , to see which method will have the most effective anti-bacterial properties on two different strains of bacteria (*E. coli, S. epidermidis*).

Research and Experimental Methodology:

For this project I will rely on secondary research. Which includes research articles and information from websites on the *S. frutescens* , extraction methods of antibacterial compounds and statistics regarding specific diseases affecting South Africa. I will also be doing primary research such as using different extraction methods to extract the antibacterial compounds from *S. frutescens* . I will testing the extracts on two different strains of bacteria.

Limitations

The limitations that I would face in my research task would be the reliability of the research articles I used with regards to the *S. frutescens.* The strains of bacteria that I'm using are harmful to human beings.

Review of Literature

Source 1:

Title:

A review of the taxonomy, ethnobotany, chemistry and pharmacology of Sutherlandia frutescens (Fabaceae).

Authors:

B-E. van Wyk, C. Albrecht

Year of publication:

2008

Summary:

The article is a review of many different articles on *S. frutescens.* The article focuses on the chemistry and ethnopharmacology of *S. frutescens.* It names the ailments that *S. frutescens* is used to treat ailments such as urinary tract infections and HIV. It's also used as an antibacterial and anti-inflammatory. Its has been shown that S. frutescens has been widely used as a medication by various groups in South Africa particularly the in the Western Cape.

Validity:

This article is review of many different articles and most of the information is derived from other articles concerning *S. frutescens* and its properties and uses.

Reliability:

This article is from the Journal of Ethnopharmacology, which is published on the journal publishing site elsevier. com. The journal was also reviewed by a board of editors from many different countries.

Evidence use to support conclusion:

The leaves of the Sutherlandia frutescens have antibacterial properties. Recent studies on this plant have mostly focused on the anti-cancer, anti-HIV, anti-diabetic, anti-inflammatory, anti-oxidant, analgesic and antibacterial activities.

Usefulness:

The article describes the many uses and properties of *S. frutescens* such as it’s antibacterial properties, it’s anti-inflammatory and its anti-HIV properties. The article also goes over the many uses of S. frutescens ov

Limitations:

The article doesn’t elaborate on much on the antibacterial activities of *S. frutescens* The article doesn’t show methods of extracting *S. frutescens.*

Author Credentials:

B-E. van Wyk is a professor at the University of Johannesburg and teaches undergraduate plant taxonomy, postgraduate taxonomy, systematics, chemosystematics of African plant families, medical plant chemistry and ethnobotany.

Source 2:

Title:

Antibacterial and Antioxidant Activity of Sutherlandia frutescens (Fabaceae), A Reputed Anti-HIV/AIDS Phytomedicine

Authors:

David R. Katerere† and Jacobus N. Eloff*

Year of publication:

2005

Summary:

The article describes the extraction methods that were used to extract *S. frutescens* The article also describes the how the different extracts where tested on different strains of bacteria. The article is about the antibacterial and antioxidant activity of *S. frutescens* . The second method of extraction produced a greater yield than the first method of extraction.

Validity:

The first extraction method used five grams of a commercially available leaf sample of Sutherlandia frutescens (Sutherlandia/ Unwele®). The Sutherlandia frutescens (Sutherlandia/ Unwele®) sample was consecutively extracted three times using different substances, first with Hexane (coded SF-H), then by dichloromethane (DCM) (SF-D), then by acetone (SF-A) and finally by ethylacetate (SF-E). The second method consisted of splitting a leaf sample of a Sutherlandia frutescens into three portions weighing 5g each. The portions where portions where extracted separately twice with acetone, ethanol and water. Each extract was then dried using a rotary evaporator and weighed. The aqueous extract was then freeze dried.

Reliability:

S, frutescens was extracted using two different extraction methods.

Evidence use to support conclusion:

The total yield of all four solvents in the first method of extraction was 10. 5%. In the second extraction method, acetone extracted 5. 6%. Ethanol extracted 12. 6% while eater extracted 17. 2%.

Usefulness:

It’s useful as it gives methods to extract the active ingredient from the plant.

Limitations:

The article doesn’t give a testing method that I can easily perform at school.

Author’s Credentials

David R. Katerere†: Specialist Scientist at SA MRC, Visiting scientist at Scynexis, visiting scientist at UNINA, trainee Pharmacist at Drug Tech Pharmacy, Chief Bioanalyst at PAREXEL, Postdoc at University of Pretoria

Jacobus N. Eloff*: Gold Medal for Science for Society Academy for Science of South Africa (September 2012), Gold medal of the South African Academy for Science and Art is awarded for Scientific and Technological Achievement, Bronze medal from the International Horticultural Society (December 2008) in recognition of the organising the World Conference on Medical and Aromatic Plants.

Source 3:

Title:

Five Ochna species have high antibacterial activity and more than ten antibacterial compounds

Authors:

Tshepiso J. Makhafola1

Jacobus N. Eloff1

Year of publication:

2011

Summary:

The article is about the antibacterial activities of five Ochna species. Leaf samples where extracted using different mediums from the leaf. The extracts were tested against various strains of bacteria.

Validity:

The dried leaf powder was extracted with 20mL of acetone.

The solution was then shaken in 50 mL centrifuge tubes and centrifuged for 15 minutes at 4000 rpm. The extracts were decanted through into glass vials through filter papers and the solution was concentrated to dryness with a stream of cold air.

Only clean and dry leaves were selected, the selected leaves had no blemishes or dirt. The leaves were not washed with water as the water would possibly extract some water-soluble compounds, and to limit the posibilty of fungal growth on the leaves due to the moisture left on the surface due to the water. The leaves were dried at room temperature in the dark. The leaves were then made into a fine powder, with the particles being less than 1 mm in diameter. The leaves were then stored in sealed glass bottles in the dark to reduce chemical changes in the compounds present in the leaves.

Reliability:

There were no competing interests the article.

Evidence use to support conclusion:

The percentage yield in acetone between the five species was: O. gamostigmata (8%), followed by O. pulchdra, (7. 5%), O. serullata (7%) O. pretorienses and O. natalitia ((2. 5%)

Usefulness:

This article shows different extraction methods and it also gives a suggestion to which extraction method and solvent worked the best to extract the particular compounds. It provides detailed images, tables and graphs which makes it easier to view the data that was collected.

Limitations:

Only gives information about on genus of plant (Ochna) and there is no information of *S. frutescens.*

Author's Credentials

Kobus (Jacobus N) Eloff: Gold Medal for Science for Society, Eskom award for capacity development, Gold medal of the South African Academy for Science and Art is awarded for Scientific and Technological Achievement, Gold Medal for Botany

Tshepiso Makhafola: Attended the University of Pretoria from 2008-2010. He has skills and expertise in research, molecular biology and biotechnology.

Source 4:

Title:

Influence of Sutherlandia frutescens extracts on cell numbers, morphology and gene expression in MCF-7 cells

Authors:

B. A. Standera, S. Maraisa, T. J. Steynberga, D. Theronb, F. Joubertc, C. Albrechtd and A. M. Jouberta

Year of publication:

2007

Summary:

The article is about the influence of *S. frutescens* on cell numbers, morphology and gene expression in MCF-7 cells. An extraction was made our of small twogs and leaves, the solution was then filtered. It was demonstrated that ethanolic extracts of S. frutescens inhibited multiplying of MCF-7 mammary adenocarcinoma cells.

Validity:

Dulbecco’s minimum essential medium eagle (DMEM) with Glutamax™ (Gibco BRL, USA)

• Trypsin–EDTA

• Crystal violet DNA stain was used to determine the number of cells. (Spectrophotometrically)

• Heat inactivated fetal calf serum (FCS) was used to culture the MCF – 7 human breast cell line.

• Penicillin was used to culture the MCF – 7 human breast cell line.

• Streptomycin was used to culture MCF – 7 human breast cell line.

• Sterile cell culture flasks

• 96-well plates where used to house the culturing cells.

• MCF-7 human breast a denoma carcinoma cell line were cultured in DMEM

• Cell Morphology: Two hundred and fifty thousand

MCF-7 cells were put onto heat-sterilized coverslips in well plates and they were exposed to 1. 5 mg/ml of Sutherlandia Frutescence extract for periods of 24, 36, 48, and 72 hours at 37°C cells where counted using a microsceope.

Reliability:

Sterile culture flasks and well plates where used, the cultures where kept at a constant temperature of 37°C and in a humidified atmosphere with 5% CO2, the specimens of Sutherlandia frutescens were air dried in the shade in the area of Murraysburg in the Karoo, to reduce the chance degradation of the specimens. The specimens where identified as Sutherlandia frutescens by the botany and biotechnology department at the university of Johannesburg.

1 gram of Sutherlandia frutescens was mixed with 10ml of 70% ethanol to produce a stock solution. After the extraction of the Sutherlandia frutescens it was centrifuged to remove any debris and then it was filtered twice to obtain a purified 100mg/ml stock solution.

The cells where cultured for 24 hours. Vehicle controles where used prove the effectiveness of the Sutherladnia frutescens.

The results that were obtained were statistically analysed for significance using analysis of variance factor model. This was then proceeded by a two-tailed Student’s t-test.

Evidence use to support conclusion:

The ethanol extracts of the Sutherlandia frutescens inhibited the growth of the MCF-7 mammary adencarcenoma cells of the period of 72 hours. 1. 5 mg/ml of the Sutherlandia frutescens ethanol extract was statistically found to reduce 50% of the growth of MCF-7 cell over 24 hours when compared to the vehicle-treated control.

Usefulness:

It shows different methods of extracting the Sutherlandia frutescens and different substances used to extract the plant. It also gives results that have been statistically proven.

Limitations:

There aren’t any tests to prove its antibacterial effectiveness.

The article doesn’t mention the chemical compounds present in the plant that prove it’s effectiveness.

Author’s Credentials

B. A. Stander: Department of Physiology, University of

Pretoria, P. O. Box 2034, Pretoria 0001, South

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S. Marais: Department of Physiology, University of Pretoria,

P. O. Box 2034, Pretoria 0001, South

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T. J. Steynberg: Department of Physiology, University of Pretoria, P. O. Box 2034, Pretoria 0001, South Africa

D. Theron: ACGT Microarray Facility, University of Pretoria, 0002 Pretoria, South Africa

F. Joubert: Bioinformatics and Computational Biology Unit, University of Pretoria, 0002 Pretoria, South Africa

C. Albrecht: Cancer Association of South Africa, P. O. Box 2121, Bedfordview 2008, South Africa

A. M. Joubert: Department of Physiology, University of Pretoria, P. O. Box 2034, Pretoria 0001, South Africa

Source 5:

Title:

Antibacterial Activity of Leaf Extracts from Combretum micranthum and Guiera senegalensis (Combretaceae)

Authors:

Stefano Banfi, Enrico Caruso, Viviana Orlandi, Paola Barbieri, Serena Cavallari, Paolo Viganò, Pierangelo Clerici and Luca Chiodaroli

Year of publication:

2014

Summary:

Guiera senegalensis and Combretum micranthum lwaves were used and tested on for the presence of antibacterial compounds.

Five solvents were used to extract the plant material; the solvents were used in increasing polarity. Escherichia coli C1a and Staphylococcus aureus MSSA were used to test the antibacterial effectiveness of the plants. A bioautographic method was used to monitor the antibacterial activity of the plants extracts throughout the purification steps. The Minimum Inhibitory Concentration and Minimum Bacterial Concentration of the most purified and active plant extracts were evaluated at the end of the procedure.

Validity:

Dry leaves extraction procedure: Whole leaves of C.

micranthum and G. senegalensis, were dried immediately after obtaining them from the plant in a local drying room at 40°C.

The dried leaves were then sent to Varses. Dried whole leaves weighing 100g were poured in a 2. 5 L bottle and treated with 600ml of cyclohexane

(least polar solvent). After a period of 24 hours the leaves were separated from the solvent by means of a Buckner funnel. This procedure was repeated using progressively more polar solvents: toluene, acetone, EtOH and water respectively.

Agar diffusion assay: Between 4-5 isolated colonies of each strain were collected and resuspended in 5ml of PB. It was then put onto its respective solid growth medium by means of a sterile cotton swab. The plates were incubated at 37°C for a set amount of time required for each microorganism. The antibacterial effect of the extract was measured by measuring the growth inhibition halo. Pictures if the inhibition halos were taken using a camera to document the findings.

Reliability:

Incubation temperature was kept constant at 37°C. Evidence of the inhibition rings were taken by means of a photo camera and those images were later analysed. A fair test was performed as four different methods of extraction where used, each with increasing polarity.

Evidence use to support conclusion:

Cm4-P showed good activity against S. aureus and S. xylosus.

Cm4-P showed some activity against Gram negative strains. Gs2-Paq was found to be more active against the Gram positive strians compared to Cm4-P.

Usefulness:

Gives an example of how an extraction could be done by ordering the solvents according to polarity. It shows how the inhibition rings can be measured and analysed i. e. By means of taking photographs.

Limitations:

The article doesn't show extraction methods and testing methods for *S. frutescens*

Author's Credentials

Stefano Banfi: Degree in organic chemistry in February1980 at the University of Milan, Assistant Professor in Organic Chemistry.

Enrico Caruso: Graduated with a degree in organic chemistry in October 1998 from the University of Milan, Assistant Professor in Organic Chemistry,

Viviana Orlandi: 1995: Degree in Biological Sciences, University of Milan discussing a thesis on " Expression of oppioid receptor in primary coltures of murine cortex neurons: trasduction signal pathway and interaction with glutamate receptors". Member of the Italian Society for General Microbiology and Microbial Biotechnology (SIMGBM).

Paola Barbieri: 1980: Degree in Biological Science at the University of Milan, Institute of Genetics. Member of the American Society for Microbiology (ASM)Member of the Italian Society for General Microbiology and Microbial Biotechnology (SIMGBM).

Serena Cavallari:

Paolo Viganò: Degree in Biological Sciences; Postgraduate Diploma in Microbiology, Doctor of Biological Sciences; Specialist in Microbiology

Luca Chiodaroli:

Conclusion

Source 1 deals with the general usage of *S. frutescens* as a medicinal plant in South Africa. Source 2 deals with the antibacterial and antioxidant properties of *S. frutescens* . It also shows extraction methods and bacterial testing methods. Source 3 shows the antibacterial activities of the *Ochna* species of plants. This source gives an indication of what types of bacteria that need to be used for testing the antibacterial activities of the *S. frutescens.* Source 4 is about the influence of *S. frutescens* extract on MCF-7 cells. It has a good indication of an extraction method that can be used. Source 5 is about the antibacterial activity of leaf exracts from *Combretum micranthum* and *Guiera senegalensis* . It gives an example of an extraction method that can be used for *S. frutescens.* All the sources deal with extraction method that can be used for certain plants. Not all the articles deal with the extraction methods and testing of *S. frutescens.*

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Fritz Lherisson, F. (2014). *South Africa* . [online] Unaids. org. Available at: http://www. unaids. org/en/regionscountries/countries/southafrica/[Accessed 16 May. 2014].