EXTRACTION OF MURORO *(Annona senegalensis)* AND MUHACHA *(Parinari curatellifolia)* PLANTS AND THEIR EFFICACY ON PUFF ADDER HAEMOTOXINS.

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Abstract

Snake envenomation in Zimbabwe from Puff Adders as a health issue has caused high mortality rates. Currently, Zimbabwe import snake antivenom antisera for vipers and other types of snakes from outside countries like DRC, South Africa and India. Some of these antivenom also lack efficacy against targeted snake species. Snake venom antisera use have limitations like failing to neutralise the puff adder strong heamotoxins, high cost and unavailability, inaccessibility for the rural patients, the storage conditions, short expiry period and dosage problems associated hypersensitive reactions which obligates further medical attention, contribute to mortality rates in Zimbabwe. For the first time Annona Senegalensis and Parinari Curatifollia are to be studied on their efficacy against puff adder haemotoxins.

Fresh parts of Muroro *(Annona Senegalensis)* and muhacha *(Parinari Curatifollia)* were collected at Scientific and Industrial Research and Development Centre and air dried at room temperature. All the plants were then extracted using water, ethanol and methanol.

Muroro leaves, muroro root bucks and muhacha sterm bucks were extracted for phytochemicals using water, methanol and ethanol. All plant extracts were incubated at mesophilic temperature. Muroro leaves managed to show efficacy to venom haemotoxins within few hours in methanol, ethanol and water extracts with highest in methanol extracts. Muroro roots extracts was on average than other extracts. Muhacha sterm bucks show a very low efficacy in all extracts.

KEYWORDS: Snakebite, snake venom, antivenom, Puff adder

Introduction

Snakebites comprise a serious health problem in several countries due to their global incidence, which exceeds 2.5 million per year, and the elevated number of victim fatalities *(according to WHO)*. To counteract envenomations, antivenoms from horse serum have been used regularly for more than a century. Apart from side effects including anaphylactic reactions, antivenoms are not able to efficiently neutralize and repair local tissue damage, which contributes to increasing the severity and morbidity observed in patients. This fact, in turn, may be responsible for economic hardship, particularly in rural populations of developing countries like Zimbabwe.

In Zimbabwe, bites are primarily due to the venomous species of the families Elapidae and Viperidae. 90% of the bites are being caused by Puff Adder *(bitis arietans)* that contains strong haemotoxins among other vipers *(according to snake world, Zimbabwe)*. Snake envenomination cause a major problem mainly in rural areas and newly resettled areas due to lack of basic health facilities, unavailability, inaccessibility, high costly poor storage conditions of snake antivenom, dosage problems associated hypersensitive reactions which obligates further medical attention . Lack of readily available transportation, in rural areas, many victims fail to reach hospital in time, some even die before reaching hospital.

Puff adder venom is cytotoxic with strong Haematoxic and some cardiotoxic effects and large volumes are produced. It causes severe pain, swellings in the bitten limb, hemorrhages and nausea. Death is caused by secondary effects, such as kidney failure, due to severe swellings. The long fangs (12 - 18 mm) assure that the venom is injected rather deeply

In most parts of the country were venomous snakes occur, numerous plant species are used to treat snake bites. Generally water or an aqueous, ethanol, methanol extract is prepared for the plant parts. Topical application of the plant or its sap onto the bitten area or chew leaves, buck or drinking plant extract or injecting the extract are some procedures to counter current snake venom activity. Medicinal plants have been used for centuries as remedy for human diseases because they contain the compounds of therapeutic values. This are of prime importance to the health of individuals and communities and the medicinal values of these economically important plant species is due to the presence of certain secondary metabolites. Medicinal plants and their extracts are used in traditional treatments of various diseases. Plants are used medicinally in different countries and are the source of potential and powerful drugs. Plants produce a diverse array of bioactive molecules, making them a rich source of diverse type of medicines. The presence of diverse phytochemicals suggested that the secondary metabolites vary widely which probably is responsible in conferring a wide spectrum of biological activities. The successive extraction using solvents of varying polarities can maximize the exploitation of diverse bioactive compounds. Medicinal plant contain phytochemicals which are non-nutritive plant chemicals, that have protective or disease preventive properties. Plant synthesizes natural bioactive/phytochemical (lipid, protein, starch, sugars, phenols, alkaloids, flavonoids etc.) for the normal growth and development itself

In the present work, I evaluated the efficacy of two Zimbabwean plants extracts *Annona senegalensis* leaves and root bucks and *Parinari curatellifollia* stem bucks, for the first time, against puff adder *(bitis arietans*) haemotoxins in pig blood using UV ViS Photometric mode

Materials and Methods

Fresh parts of Annona Senegalensis and Parinari Curatifollia were collected at Scientific and Industrial Research and Development Centre (SIRDC) on the 7th April 2014. The plant materials were taxonomically identified and authourised by the Herbal Department in Biotechnology Research Institute (BRI) at SIRDC. Stem bucks of Parinari Curatifollia, roots and leaves of Annona Sennegalensis were air dried in the Microbiology laboratory of Food and Biomedical Technology Institute at SIRDC until all the water molecules evaporated and plants well dried for grinding. The plant samples were then crashed into powder at University of Zimbabwe using mechanical blender and transferred in plastic bags in preparation of extraction.

PREPARATION OF PLANT EXTRACT

Hot water extraction *(Journal of Phytology 2011, 3 (12): 10-12)*

5grams of finely dried powder plant material was taken in a beaker and 200ml of distilled water was added. The mixture was heated on a hot plate with continuous stirring at 30-40^{o}C for 20 minutes. Then the water extract was filtered through filter paper and the filter was used for qualitative phytochemical analysis. The water extracts was kept in refrigerator when not in use.

Solvent Extraction *(Journal of Phytology 2011, 3 (12): 10-12)*

Maceration

5grams of finely dried powder plant material was taken in a 250ml volumetric flask and 200ml of 70% ethanol 90% methanol where added separately in 3 samples and kept for 24 hours. After maceration the extracts where filtered using filter paper and concentrated to dryness using water bath 70^{o}C for 4 hours.

Qualitative phytochemical analysis *(Journal of Phytology 2011, 3 (12): 10-12)*

Muroro leaves and root bucks and muhacha sterm bucks where tested for the presence of bioactive compounds that are capable of neutralising venom haemotoxins.

Bioactive components namely:

- Saponins
- Flavonoids
- Glycosides
- Steroids
- Terpenoids
- Akaloids

Test for flavonoids (shinoda test)

0.2 grams of plant extract were mixed in separate test tubes and then dissolved 20%NaOH and 32%HCL. The yellow solution turns colourless showing the presence of flavonoids.

Test for saponins

Crude extracts were mixed with 5ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was considered as an indication for the presense of saponins

Test for Glycosides (salkowski"s test)

Crude extract was mixed with 2ml of chloroform. Then 2ml of concentrated $H_2SO_4$was added carefully and shaken gently. A reddish brown colour indicated the presenses of steroidal ring i.e., glycon portion of the glycoside

Test for steroids

Crude extract was mixed with 2ml of chloroform and concentrated H_2SO_4 was added sidewise. A red clour produced in the lower chloroform layer indicated the presense of steroids.

Test for tepenoids

Crude extracts were dissolved in 2ml of chloroform and evaporated to dryness. To this, 2ml of concentrated H_2SO_4 was added and heated for about 2minutes. A greyish colour indicated the presense of terpenoids

Testing the efficacy of plant extracts

Materials

- Pig blood
- Puff Adder venom
- UV-VIS
- Plant extracts
- 11 steral test tubes
- Incubator
- Test tube rack
- SDS positive control
- 11 Purple to test tube(EDTA)

Methodology

Fresh Blood was collected aseptically from Colcom Company into 5ml purple top test tubes treated with EDTA

Positive control 0.1% SDS or 5ml Blood and 0.2µl puff adder venom
Negative control 5ml Pig blood

Preparation of 0.1% SDS from 20% SDS

$C_1V_1=C_2V_2$

C1=20%
V1=?
C2=0.1%
V2=50ml

$20 xV_1 =0.1 x 50$
$20V_1 = 5$
$V_1 = 5/20$
$V_1 =0.25$

0.25ml will be added into 50ml and distilled water will be added to the mark

NB: SDS will continually be added into the test tube to insure 100% haemolysis of RBC

%haemolysis

Is the percentage which shows the lysis of red blood cells, the higher the percentage value the higher the lysis of red blood cells. It is calculated as follows

$$\frac{At-An}{Ac-An} \times 100$$

At- absorbance of tests sample
An- absorbance of negative control
Ac –absorbance of positive control

Results

Results for plant phytochemical analysis

Key

MRB- Muroro Root Bucks
ML- Muroro Leaves
MSB- Muhacha Sterm Bucks
+ Presence
- Absense

100% WATER EXTRACTS

Plant compounds	MRB	ML	MSB
Flavonids	-	+	+
Saponins	+	+	+
Glycosides	+	+	+
Steroids	+	+	-
Terpenoid	+	+	+

70%ETHANOL EXTRACTS

Plants Compunds	MRB	ML	MSB
Flavonids	+	+	+
Saponins	-	+	+
Glycosides	+	+	-
Steroids	+	+	+
Terpenoid	+	+	+

90%METHANOL EXTRACTS

Plants Compunds	MRB	ML	MSB
Flavonids	+	+	+
Saponins	+	+	+
Glycosides	+	+	-
Steroids	+	+	+
Terpenoid	+	+	+

Results for efficacy of plant extracts

Key
T--- Transmittance
ABS---Absorbance

Blank -100% SDS in blood sample

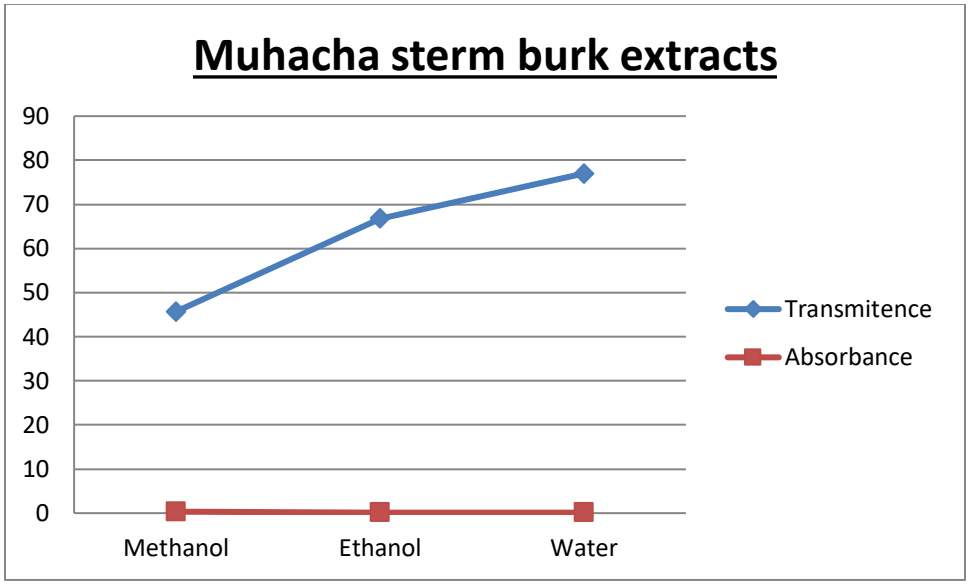
Transmittance (blank sample) = 99.7% (T%)
= 0.0013(ABS)

Blood and Puff adder Venom = 99.6 (T%)
= 0.0026 (ABS)
Pig Blood = 2.076 (T%)
= 1.682 (ABS)

SOLVENTS	SAMPLES	Transmitance%	Absorbance	%haemolysis
WATER	MRB	58.9	0.23	86%
	MSB	77.0	0.11	93%
	ML	40.0	0.39	76%
70%ETHANOL	MRB	42.3	0.37	78%
	MSB	60.3	0.21	87%
	ML	23.1	0.63	62%
90%METHANOL	MRB	33.4	0.45	71%
	MSB	30.0	0.52	69%
	ML	15.6	0.80	28%

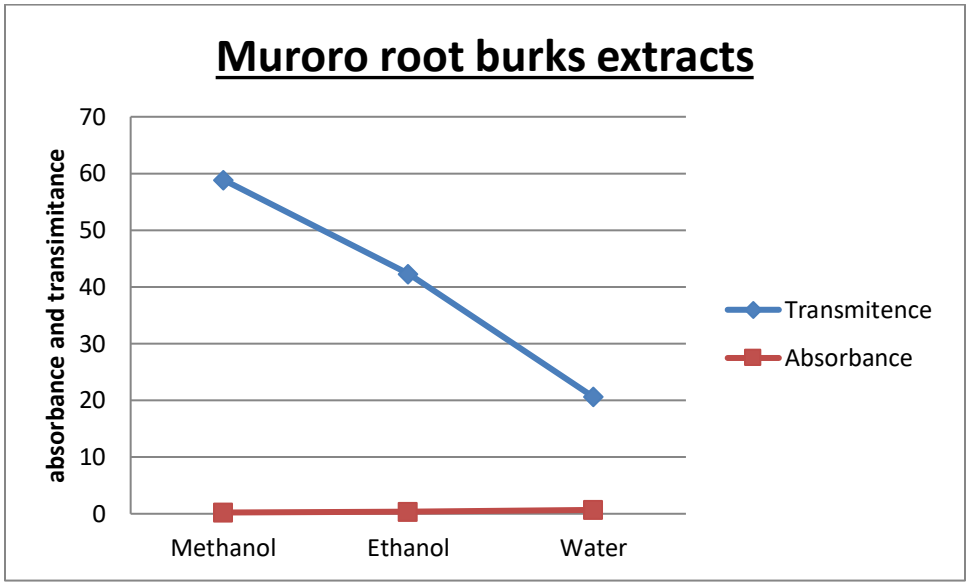
Muhacha sterm burk extracts

	Transmittance	Absorbance
Methanol	45.7	0.34
Ethanol	66.8	0.17
Water	77.0	0.11



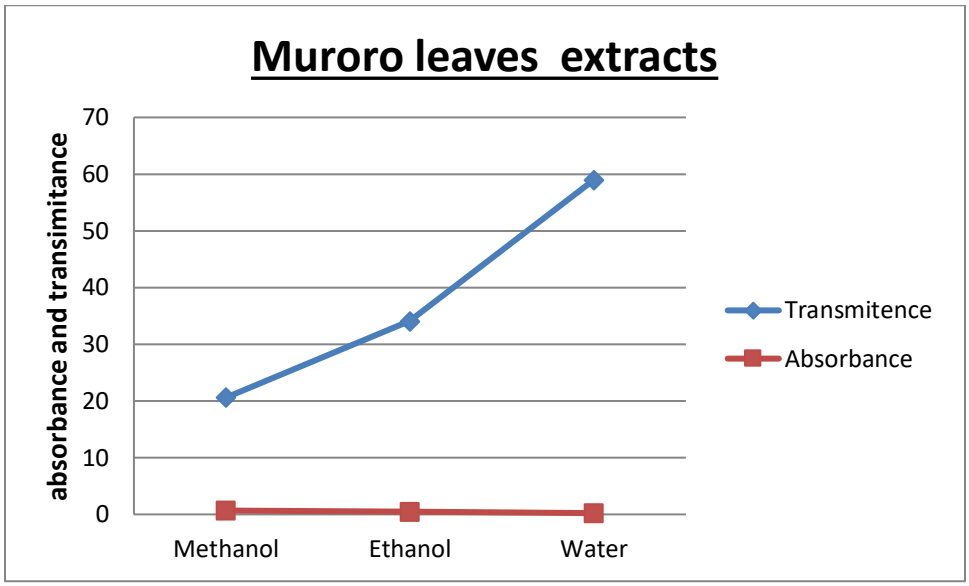
Muroro root Burks extracts

	Transmittance	Absorbance
Methanol	58.9	0.22
Ethanol	42.3	0.37
Water	20.6	0.68



Muroro leaves extracts

	Transmitence	Absorbance
Methanol	20.6	0.68
Ethanol	34.1	0.46
Water	59.0	0.23



Discussion

Snake bite remains a public health problem in many countries even though it is difficult to be precise about the actual number of cases. Medicinal plants have been used for many years to treat a great variety of diseases including envenomations by animal bites.

Therefore, experimental validation of the traditional use of plants is a necessary step in the development of low-cost phytotherapeutic agents. Many plants are recommended in some countries like India. The present investigation identified Muroro leves to have significant antihaemotoxic activity against *Puff adder* venom.

Phytochemical analysis conducted on the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities. Analysis of the plant extracts revealed the presence of phytochemicals such as phenols, tannins, flavonoids, saponins, glycosides, steroids, terpenoids, and alkaloids.

Their activity is probably due to their ability to complex with extracellular and soluble proteins. The plant extracts were also revealed to contain saponins which are known to produce inhibitory effect on inflammation . Other studies reviewed that saponins has the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness. The results obtained in this study thus suggest the identified phytochemical compounds may be the bioactive constituents and these plants are proving to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit.

Muroro leaves manage to show efficacy to venom haemotoxins within three hours in methanol, ethanol and water extracts with highest in methanol extracts. The percentage haemolysis was as low as 28% in methanol extracts and high as 76% in water extracts. Muhacha sterm bucks shows a very low efficacy in all extracts. The percentage haemolysis was as low as 69% in methanol extracts and as high as 93% in water extracts. Muroro roots extracts was on average than other extracts. The percentage haemolysis was as low in methanol extracts of 71% and high in water extracs at about 76%.

Methanol, its polar solvent, whilst ethanol exhibit both polar and non-polar charecteristics, and water is polar. Plants extracts dissolved best in polar solvents and showed an efficacy ability to haemotoxins, whilst in other solvents the efficacy was quite good than in methanol.

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