

Toxicity and Antiviral Activities of Some Medicinal Plants Used by Traditional Medical Practitioners in Zimbabwe

Deniz Iklm Viol¹, Lameck Shoriwa Chagonda^{1*}, Sylvester Rodgers Moyo²,
Ali Hikmet Mericli³

¹School of Pharmacy, College of Health Sciences, University of Zimbabwe, Harare, Zimbabwe

²Faculty of Health and Applied Sciences, Namibia University of Science and Technology, Windhoek, Namibia

³Department of Pharmacognosy, School of Pharmacy, University of Istanbul, Istanbul, Turkey

Email: *chagonda@medic.uz.ac.zw

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Abstract

Genital herpes, usually caused by Herpes Simplex Virus type-2 (HSV-2), is the commonest sexually transmitted disease especially amongst rural women in Southern Africa including Zimbabwe. This predisposes them to HIV/AIDS infection, cancer and opportunistic infections (OIs). Current antiviral treatments are often cytotoxic and/or ineffective. This motivates active research to find alternative safer drugs or lead drugs from traditional medicinal sources. Twenty six (26) methanol extracts from commonly used and often endangered plant species (14) used by communities and traditional medical practitioners for treating illnesses and sexually transmitted diseases from 5-selected districts of Zimbabwe were investigated for toxicity by Brine shrimp lethality test (BSLT) and by 50% Cytopathic effect on VERO cultured cells. The extracts were also tested for antiviral activity against Herpes Simplex Virus-2 (HSV-2) by the End Point Titration Technique (EPTT) and Neutralisation Test (NT) on VERO cells. Results from the BSLTs ranged 66.66 - 4304 µg/ml; 50% Cytopathic effect from 19.53 - 312 µg/ml whilst the NT ID₅₀ values ranged from 10.41 - 125 µg/ml. The antiviral EPTT reduction factor (RF) was 1 - 10⁴ with 13 extracts showing RF ≥ 10³. All the plant extracts had moderate to high toxicity (LC₅₀, 789 - 66 µg/ml) in the BSLT. Six extracts had LC₅₀ values greater than 1000 µg/ml. All 26 extracts were cytotoxic with CC₅₀ values < 500 µg/ml of which 19 were more toxic CC₅₀ < 100 µg/ml. Nine extracts had *in vitro* therapeutic indexes ≥ 3.7. *Cassia abbreviata*, *Dichrostachys cinerea* and *Hypoxis hemerocallidea* had therapeutic indexes (TI) 7.5 - 15.0. The more active plant extracts were from roots and root tubers. The results confirm the rationale for the use of traditional medicinal plants by traditional medical practitioners for treating various diseases and could bring awareness for their better use and improve

*Corresponding author.

conservation. The results also provide an opportunity to develop more efficacious drugs by isolating lead compounds and determining their mode of action.

Keywords

Medicinal Plants, Toxicity, Antiviral Activity, Herpes Simplex Virus-2, Zimbabwe

1. Introduction

Third world countries are often endowed with rich flora and fauna which are put to good use in their traditional medical practices. Zimbabwe has over 5000 plant species of which 500 are established in Traditional Medicine (TM) since being legalised in 1981 through an Act of Parliament [1]. Traditional Medical Practitioners (TMPs) treat common illnesses and chronic diseases with claims to treat HIV/AIDS and opportunistic infections (OIs); this is not uncommon in Africa as a whole. Medicinal plants (MPs) still play a central role as sources of drugs for drug development into modern medicines and are still the mainstay for TMPs and for Complementary and Alternative Medicines (CAM) [2]-[4]. The combined effect of poverty and unemployment, civil unrest, land hunger and disease prevalence in developing countries has pushed more and more people towards the traditional medical practice. With over 55,000 registered TMPs in Zimbabwe alone, the protection of the 500 medicinal plants identified as playing a significant role in the traditional practice becomes a problem for conservationists [1]. The ever increasing demand for more land for agriculture and land reforms, human settlements, urban and industrial expansion has put many African governments on the back foot as they seek to address these challenges. Governments are therefore challenged to add value to MPs through research to achieve the multiple goals of conservation awareness and health improvement for the poor. Through technical initiatives, protocols, and/or encouragements, research into traditional medicine and medicinal plants is being pursued vigorously [5]. In Zimbabwe, a study was set up with the help of GEF/UNDP/MET (Ministry of Environment and Tourism) to carry out scientific laboratory studies on 50 extracts from 26 medicinal plants commonly used by communities to assess their conservation status in five selected districts from two regions of the country and to raise awareness of their importance and promote their sustainable use. Subsequently, we reported the antioxidant properties of extracts from these plant species [6] [7] as well as their antimicrobial and phytochemical properties [8] [9]. The laboratory studies confirmed the rationale for their use in traditional medical practice in line with other studies elsewhere to evaluate TMs targeted at finding alternative safer drugs to treat modern illnesses and to overcome growing resistance to infective agents. Our continuing research is now focused on selective studies and value addition through identifying active principles and action mechanisms [10] [11]. The HIV/AIDS virus still remain a threat to mankind and the current report presents the toxicity and antiviral properties of 14 plant species used in traditional medical practice which could have a bearing on the development of potential antiviral and anticancer drugs. This report highlights some of the key findings of the preliminary laboratory studies on toxicity and antiviral activities in the pilot study [12].

2. Materials and Methods

2.1. Ethnobotanical Surveys and Plant Materials

Ethno-botanical surveys on traditional medicinal plants used for treating common ailments, those most traded and threatened were carried out in five districts: Bulilima, Chimanimani, Chipinge, Mangwe and Matobo and the plants identified and authenticated by the National Herbarium and Botanic Gardens, Harare, as previously reported [8]. The plant materials roots, leaves, twigs, tuber, bark or whole, were dried, finely ground and stored.

2.2. Preparation of Extracts for Bioactive Tests

Selected plant parts were powdered (30 g), macerated in methanol (200 ml) [13], filtered and the filtrate evaporated off under reduced pressure, freeze dried and stored at -20°C . Part of the lyophilised extracts were dissolved in DMSO (10 mg/ml), filtered under aseptic conditions and stored for further bioactive use at -20°C .

2.3. Brine Shrimp Bioactivity Testing

Bioactivity testing using the Brine Shrimp (*Artemia salina*) Lethality Test (BSLT) method was carried out in triplicate with different concentrations of the sterilized plant extracts in brine solution (10 µg/ml - 1000 µg/ml) and the percentage lethality of the nauplii determined [14]. The concentration of the extract that kills 50% of the shrimps, “Lethal Concentration₅₀” (LC₅₀) values were recorded using *Nerium oleander* as the positive control by GraphPad Prism 5.0 linear regression and Pearson’s two tailed analysis for 95% confidence limits (95% CI) [15].

2.4. Cytotoxicity and Antiviral Activity of the Plant Extracts

The lyophilized methanol extracts were dissolved in DMSO to a concentration of 10 mg/ml. Vero cells (African green monkey kidney) (Highveld Ltd., South Africa) were grown and maintained in essential growth medium supplemented with 5% foetal calf serum and incubated under 5% carbon dioxide at 37°C. The confluent cells were removed and trypsinated with phosphate buffered saline solution to achieve a cell concentration of $1 - 2 \times 10^4$ cells per well for VERO cells.

Herpes simplex virus 2 (HSV-2) (Highveld Ltd., South Africa) was propagated on Vero cells and the recovered virus suspension (1 ml) diluted by infecting Vero confluent monolayers grown on microtitre plates with 0.1 ml of serial tenfold dilutions of the virus suspension quadruplicated and observed for 7 days for cytopathic effect (CPE). The HSV-2 virus titre was obtained from the 50% tissue culture infections dose per ml (TCID_{50/ml}) [16].

Cytotoxicity of the different plant extracts that could cause non-specific cytopathic effect (CPE) on confluent Vero cells in growth medium was determined by using 100 µl of serial two fold dilution of the plant extracts onto confluent Vero cells on microtitre plates in quadruplicate. The 50% cytotoxicity concentration (CC₅₀) was defined as the plant extract concentration causing 50% CPE compared to uninfected cells [16]. The maximum nontoxic dilution (MNTD) was taken as the next dilution after the TCID₅₀ of the plant extract and used for the antiviral assays.

2.5. Antiviral Assays

Antiviral assays were carried out using the Neutralization test (NT) to determine non-cytotoxic concentration (ID₅₀) that inhibits/protects 50% of the monolayer cells against destruction by the virus compared to uninfected cells using the Spearman-Kärber formula [17] and by the End point titration technique (EPTT) [18]. In the EPPT assay, confluent monolayer Vero cells were grown on 96-well microtitre plates with the medium removed from the wells. 0.05 ml (MNTD) of the plant extract and 0.05 ml of growth maintenance medium followed 1 h later by 0.05 ml of tenfold HSV-2 serial diluted virus suspension and the mixture incubated at 37°C. Controls consisted of Vero cells infected with virus and not treated with plant extract and virus uninfected and untreated cells. All tests were compared with a positive control, acyclovir (Sigma). The antiviral activity was determined as the reduction factor (RF) of the viral titre: the ratio of virus titre in the absence and presence of the plant extract. A promising antiviral has a RF $\geq 10^3$ [13] [18].

3. Results and Discussion

3.1. Brine Shrimp Bioactivity Testing

The bioactivity of the plant extracts using the Brine shrimp lethality test (BSLT) indicated that plants in this category were relatively toxic with 15 of the 26 extracts recording LC₅₀ < 500 µg/ml (Table 1). Only 6 extracts had LC₅₀ > 1000 µg/ml. Potentially very toxic plants were *T. sericea* (LC₅₀ = 66.7 µg/ml) and *K. africana* (LC₅₀ = 117.4 µg/ml) showing greater toxicity than the positive control *N. oleander* (LC₅₀ = 141.7 µg/ml). The BSLT bioassay is widely used as a basic screening test for toxicity in prospecting for suspected biological activity in crude and isolated plant extracts from traditional folklore [4] [14] [15] [19]-[22].

3.2. Cytotoxicity and Antiviral Activity

Cytotoxicity effects for the extracts on Vero cells showed all 26 extracts had CC₅₀ < 500 µg/ml, 19 with CC₅₀ < 100 µg/ml and 12 with CC₅₀ < 50 µg/ml (Table 1) indicating potential antitumour and/or antiviral activity. Antiviral screening tests indicated some plant extracts possess potential activities to protect the cells against HSV-2

Table 1. Plants chosen for toxicity and antiviral tests.

No	Species, family name, voucher number, status	Plant part	BSLT LC ₅₀ (ug/ml)	CC ₅₀ (50% CPE) (ug/ml)	NT-ID ₅₀ (ug/ml)	TI: CC ₅₀ /ID ₅₀	EPTT (RF ^b)
1	<i>Cassia abbreviate</i> Oliv., Fabaceae (2721): Cm. Status: Cm	lf	454.93 ± 18.60	156.25	20.83	7.5	10 ²
		rt	445.72 ± 22.15	156.25	10.41	15.0	10 ³
		bk	1319.37 ± 356.63	39.06	NA	-	1
2	<i>Dichrostachys cinerea</i> (L.) Wight and Arn. Mimosaceae (32): Cm.	lf	539.39 ± 78.24	78.13	10.41	7.5	10 ⁴
		rt	4304.59 ± 685.69	312.50	83.33	3.7	10 ²
3	<i>Elaeodendron matabelicum</i> (Loes). Steedman Celastraceae (2121): Th., Endemic	rt	1012.31 ± 217.69	78.13	NA	-	1
4	<i>Elephantorrhiza goetzei</i> Harms. Fabaceae (7136): Th.	rt	356.55 ± 24.55	156.25	83.33	1.9	10 ²
5	<i>Flacourtia indica</i> (Burm. f.) Merr. Flacourtiaceae (57/62): Th.	lf	281.81 ± 26.14	78.13	83.33	0.9	10 ²
		rt	467.31 ± 39.01	156.25	125.00	1.3	10 ³
6	<i>Gymnosporia senegalensis</i> (Lam.) Loes. Celastraceae (15): Cm.	lf	789.37 ± 104.06	39.06	10.41	3.8	10 ³
		rt	2185.61 ± 872.25	78.13	20.83	3.8	10 ³
		tw	754.70 ± 182.57	19.53	15.63	1.2	10 ³
7	<i>Hypoxis hemerocallidea</i> Fisch. & Ave'-Lall. Hypoxidaceae (MTDV06): Th.	tb	735.34 ± 89.39	156.25	10.41	15.0	10 ³
8	<i>Khaya anthotheca</i> (Welw.) DC. Meliaceae (892): Cm.	bk	482.19 ± 43.49	39.06	31.25	1.2	10 ²
9	<i>Kigelia africana</i> (Lam.) Benth. Bignoniaceae (5990): Cm.	bk	262.20 ± 25.07	39.06	31.25	1.2	10 ³
		ft	117.41 ± 30.27	39.06	31.25	1.2	10 ⁴
		rt	501.35 ± 34.88	39.06	NA	-	1
10	<i>Rhuschirindensis</i> Baker f. Anacardiaceae (103/67): Cm.	lf	1023.26 ± 161.69	312.50	NA	-	1
		rt	316.60 ± 30.07	78.13	20.83	3.8	10 ²
11	<i>Sclerocarya birrea</i> (A. Riich.) Hochst. Anacardiaceae (3114): Th, Cm.	bk	1112.37 ± 210.04	39.06	20.83	1.9	10 ³
12	<i>Securidaca longepedunculata</i> Fresen. Polygalaceae (264/59): V Th.	rt	351.89 ± 35.70	78.13	20.83	3.8	10 ³
13	<i>Terminalia sericea</i> Burch. Ex. DC. Combretaceae (5): Th., endemic	lf	66.66 ± 49.31	39.06	31.25	1.2	10 ²
		rt	295.33 ± 37.19	39.06	20.83	1.9	10 ³
14	<i>Warburgia salutaris</i> (Bertol.f.) Chiov. Canellaceae (CPDV06): V Th.	lf	351.41 ± 29.58	78.13	NA	-	1
		rt	426.10 ± 55.55	39.06	31.25	1.2	10 ³
		bk	359.66 ± 14.33	19.53	NA	-	1
15	<i>Nerium oleander</i> (ref-positive control)	lf	141.67 ± 68.15		Acylor-1.50		

Plant part investigated: lf = leaf; rt = root; tb = tuber; bk = bark; Environmental status S: Cm = common; Th = threatened; VTh = very threatened; BSLT, Brine shrimp lethality test LC₅₀, lethal concentration that kills 50% of the shrimps with corresponding 95% confidence intervals (95% CI); CC₅₀ (50% CPE), plant extract cytotoxicity concentration that kills 50% tissue cells; NT, neutralisation test; NA = no activity; ID₅₀, non-cytotoxic concentration that inhibits/protects 50% uninfected cells, NA = no activity; TI: therapeutic index = CC₅₀/ID₅₀ EPPT, End point titration test. RF^b antiviral reduction factor: ratio of viral titre of control in absence of extract over viral titre in presence of extract.

induced destruction with EPTT reduction factors (RFs) $\geq 10^3$ (Table 1). *D. cinerea* (RF = 10⁴) and *K. Africana* (RF = 10⁴) were particularly active, however, most plants in the group had RFs $\geq 10^3$ which activities were also mirrored in the neutralization test (NT) ID₅₀s where acyclovir the positive control had ID₅₀ = 1.5. *G. senegalensis*, *T. sericea* and *W. salutaris* previously noted for their high antimicrobial activities [8] were also in the group of potential antiviral plants with high cytotoxicities (CC₅₀ < 50 µg/ml) and high reduction factors (RF = 10³) for some of their extracts. However, in addition to having a high reduction factor, a plant extract should also have a high therapeutic index (TI)/high sensitivity value in order to prevent cell destruction and be considered for further potential antiviral investigation. From this group (Table 1), *Cassia abbreviate* had TI values of 7 and 15 for leaf and root extract respectively, *Dichrostachys cinerea* TI = 7.5 for leaf extract and *Hypoxis rooperi* had TI = 15 for the tuber. *Kigelia* fruit showed a high reduction factor 10⁴ but low therapeutic efficacy 1.2 in line with other reports where its anti-tumour and other biological properties are highlighted [21]-[28]. Plant extracts

Gymnosporia senegalensis (Celastraceae), *Warburgia salutaris* (Canellaceae) and *Terminalia sericea* (Combretaceae) are popular plants in traditional practice. Recent studies are focusing on the toxicity, antiviral and antitumor properties of crude and isolates against different viral species especially HIV-1, HSV-1, HSV-2, anti-Dengue [29]-[38]. In general, all the plants in this study displayed both antiinfective and toxic properties (Table 1) whilst reports from phytochemical studies reflect flavonoids and condensed tannins largely responsible for the observed antiviral and anticancer activities [25]-[28] [38] [39]. Isolated fractions and derivatives can have greater toxicity and/or activity than the crude extracts, the case of artemisinin from *Artemisia annua* in the treatment of malaria [39] and the isolated phytoconstituents from *Kigelia* and *Hypoxis* species [24]-[27].

4. Conclusion

Sub-Saharan Africa still suffers the great burden for HIV/AIDS with millions infected and affected. Though prevalence has fallen, availability of antiviral drugs poses a continuing challenge. The use of TMPs is often plagued with issues of safety and/or toxicity. The antimicrobial and antiviral activities demonstrated by the plant extracts are further proof to support claims by TPs of their ability to treat more serious illness including HIV/AIDS and opportunistic infections [5]. Studies carried out from cited literature on traditional medicinal plants elsewhere have also demonstrated their potential antimicrobial and antiviral activities. Whilst the laboratory studies and biological results confirm traditional folkloric uses, their application in modern medicine has enormous challenges with respect to the standardisation of crude extracts, isolation and characterisation of key active principles and the adoption of clinical protocols gave the wide range of related plant species in different countries. This may not be helped by the lack of sponsorship for such research gave the poor state of the economies of most developing countries. In some cases, the active principles and their derivatives have proven more active than their crude extracts: artemisinin and derivatives (from Chinese *Artemisia annua*) [39] in the treatment of malaria; sitosterols, hypoxoside and rooperol (from African *Hypoxis* spp.) in the treatment of prostate cancer [25]. Likewise extracts from *D. cinerea* and *C. abbreviata* (Fabaceae), *Kigelia africana* (Bignoniaceae) and *T. sericea* (Combretaceae) should be studied for active principles, derivatives and followed by pre-clinical trials possible due to the wide spectrum of activities for isolates. The wide geographical variables make this some task but regions can adopt standards consistent with their findings from the wild where they are endemic or from propagation efforts where these are applicable. Our studies focused on local plants and reported wide scientific data which should attract more research. Such increased research activity has the potential to promote traditional medical practice by value addition to the medicinal plants used to treat patients, improve quality standards and the formulation of traditional remedies, bring awareness to the environmental status of TMPs and create opportunities for further development of TMPs into modern medicines.

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