Evaluation of traditionally used medicinal plants for anticancer, antioxidant, anti-inflammatory and anti-viral (HPV-1) activity

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Highlights:

- The anticancer activity of four medicinally used plants against cervical and epidermoid cancer.
- *S. jambos* showed 100% viral inhibition at a non-toxic concentration of 50µg/ml.
- *S. jambos* significantly inhibited cyclooxygenase-2 (COX-2) and the DPPH free radical.

- The collective data of *S. jambos* shows its potential as a chemopreventive and anticancer agent.

Abstract

The aim of this study was to determine the anticancer, antioxidant, anti-inflammatory and antiviral activities of traditionally used medicinal plants.

The extracts were tested for cytotoxicity against human melanoma (A375), epidermoid carcinoma (A431), cervical epithelial carcinoma (HeLa) and human embryonic kidney cells (HEK-293). The antioxidant and anti-inflammatory activities were also determined. *Gomphocarpus fruticosus*, *Helichrysum kraussii* and *Syzygium jambos* were selected for activity against the herpes simplex virus type-1.

The extracts exhibited low toxicity towards HEK-293 cells, and four extracts; namely *Acacia mellifera*, *G. fruticosus*, *H. kraussii* and *S. jambos*, were able to inhibit the A431 and HeLa cells with fifty percent inhibitory concentrations (IC_{50}) ranging from 34.90-56.20 µg/ml. *Arbutus unedo*, *Combretum molle*, *Dissotis princeps*, *Erythrophleum lasianthum*, *Harpephyllum caffrum*, *H. kraussii* and *S. jambos*, showed high DPPH inhibitory activity, with IC_{50} values ranging from 2.41–5.25 µg/ml. The highest antioxidant activity was seen for *S. jambos* (DPPH) and *A. unedo* (NO) respectively with greater activity than ascorbic acid. *D. princeps*, *H. caffrum*, *Leucas martinicensis* and *S. jambos*, showed high inhibition of the cyclooxygenase-2 (COX-2) enzyme with IC_{50} values ranging from 3.79-25.80 µg/ml with *S. jambos* showing the highest activity. *S. jambos* further showed the highest anti-HSV-1 activity at 50.00 µg/ml against 100$TCID_{50}$virus challenge dose.

This is the first report of the selected plants for their cytotoxicity, anti-inflammatory and viral inhibitory activity. *S. jambos* was able to show high inhibition of the HPV type-1 virus and the COX-2 enzyme.

Keywords: Anticancer, DPPH, Nitric oxide, COX-2, Herpes simplex virus, *Syzygium jambos*

Abbreviations: XTT: 2,3-Bis-(2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxyanilide salt; PGE_2: Prostaglandin E2; NO: Nitric Oxide; COX-2: Cyclooxygenase-2; DMEM: Dulbecco's Modified Eagles Medium; EMEM: Eagle's Minimum Essential Medium

1. Introduction

Traditional medicine is still used by approximately 65-80 % of the world's population in developing countries as a source of primary health care (Tag et al. 2012). The main reason for the use of traditional medicine is due to affordability, accessibility and cultural beliefs (Benzie and Wachtel-Galor, 2011). In South Africa it is estimated that approximately 27 million individuals still rely on traditional medicine. Approximately 20,000 tons of indigenous South African plants are used each year from at least 771 plant species (HST, 2015).

In this study plants were selected based on their traditional usage for the treatment of various types of cancer and/or skin ailments such as sores, wounds and infections which could lead to the development of skin or cervical cancer. Plants were also selected based on usages which could contribute towards the health of skin such as blood-cleansing and astringent properties. An infusion of *Acacia caffra* bark is used by the Zulu community of South Africa as a blood-cleansing emetic (Hutchings et al. 1996). Water extracts from the *Acacia mellifera* plant is used by a Kenyan community, known as "Kipsigis", for the treatment of various skin diseases (Mutai et al. 2008). The leaves of *Arbutus unedo*, which is commonly known as the "Strawberry tree", are traditionally used for its astringent properties (Pabuçcuoğluetal., 2003). The Tswana communities of South Africa use the fresh flowers of *Buddleja salviifolia* as a

decoction for the treatment of sores (Hutchings et al. 1996). The leaves of *Clematis brachiata* are used by many South African communities, such as Xhosas, Zulus, Sothos and Tswana's, as a soothing foot wash for cracking and blistering feet (Viljoen, 2002). *Combretum molle*, which is commonly known as the velvet bushwillow, is used by Zulu communities. A paste made from fresh or dried leaves of *C. molle* are directly applied to wounds (Hutchings et al. 1996). The roots of *Dissotis princeps* are used as a food source during times of famine. However, there have been reports of other *Dissotis* species which are used for the treatment of skin diseases (Ndjateu et al. 2014). An infusion of *Erythrophleum lasianthum* bark is used as a blood-cleansing emetic (Hutchings et al. 1996). The roots of *Euclea divinorum*, known as the "magic guarri", are extracted and used for the treatment of cancers as well as ulcers, wounds and snake bites (Prota4U, 2005). *Gomphocarpus fruticosus*, or more commonly "milkweed", is used in Ethiopia as an ointment for sores, and in Namibia a tea is made from the leaves for the treatment of skin cancer (Prota4U, 2011). Decoctions from the bark of *Harpephyllum caffrum* is used as a blood purifier and is also used as a face wash for the treatment of acne and eczema (Van Wyk, 1997). The roots of *Helichrysum kraussii* are ground and mixed with salt to treat venereal diseases, which could lead to the development of cervical cancer (Lourens et al. 2007). A decoction of the leaves or aerial parts of *Leucas martinicensis* is used for the treatment of inflammation as well as rheumatism (Chouhan and Singh, 2011). In India the "Rose apple" tree, *Syzygium jambos*, is used for the treatment of skin problems such as rashes (Zheng et al. 2011). *Tabernaemontana elegans* is used by the Zulu community of South Africa where the dried powder of the inner wall of the fruit is boiled in water and used to treat cancer (Cheek, 2010). *Warburgia salutaris* is used for the treatment of sores where the powder of the plant is applied directly (Rabe and van Staden, 1997).

In the present study, traditionally used plants were tested for their anticancer activity against skin and cervical cancer. The antioxidant and anti-inflammatory (COX-2) activities of the extracts were also determined. Selected plant extracts were evaluated for antiviral activity against herpes simplex virus type-1 (HSV-1).

2. Materials and methods

2.1 Materials

All cell lines were obtained from American Type Tissue Collection (ATCC), MD, USA and European Collection of Cell Cultures (ECACC), England, UK. Foetal bovine serum (FBS) was purchased from Separations (Pty) Ltd. (Randburg, Johannesburg, RSA). The cell culture media and antibiotics were supplied by Highveld Biological (Pty) Ltd. (Modderfontein, Johannesburg, RSA). The Cell Proliferation Kit II (XTT) for the cytotoxicity assay was purchased from Roche Diagnostics (Pty) Ltd. (Randburg, Johannesburg, RSA). The PGE_2 ELISA kit was purchased from Biocom Biotech (Pty) Ltd. (Pretoria, South Africa). All other chemicals and reagents were of analytical grade and were acquired from Sigma Chemicals Co. (St. Louis, MO, USA).

2.2 Methods

2.2.1 Extraction and collection of plant material

The plant material was collected at the Walter Sisulu National Botanical Gardens, Roodepoort; Venda, Limpopo and at the Manie van der Schijff Botanical Gardens, University of Pretoria. The plant material was identified and voucher specimens were deposited at the HGWJ Schweickerdt Herbarium, Pretoria, South Africa (Table 1). The plant material was

Table 1

Cytotoxicity, antioxidant and anti-inflammatory (IC_{50} in µg/ml) activity of traditionally used plant extracts

Plant extract	Plant part	PRU #	IC_{50}[a] (µg/ml)						COX-2[d] inhibition at 10µg/ml (%)	IC_{50} (µg/ml) COX-2
			HeLa	A431	A375	Hek-293	DPPH[b]	NO[c]		
Acacia caffra (Thunb.) Willd	Leaves	120014	185.00±0.40	132.00±0.70	>200	>200	22.30±0.32	>1000	73.79±10.00^{+}	ENS
Acacia mellifera (M. Vahl.) Benth	Leaves	078373	54.40±2.00	61.70±0.30	180.00±0.80	>200	129.00±3.00	>1000	18.72±37.00	-
Arbutus unedo L.	Leaves	120018	174.00±25	112.00±0.40	>200	>200	4.51±0.20^{+}	85.90±1.00*	47.58±21.00	-
Buddleja salviifolia (L.) Lam	Leaves	120009	>200	>200	>200	>200	37.30±1.00	>1000	46.42±19.00	-
Clematis brachiata Thunb	Leaves	120016	>200	>200	>200	>200	75.90±1.00	>1000	40.83±22.00	-
Combretum molle R.Br ex G.Don	Leaves	120015	100.00±5.00	174.00±7.00	>200	132.00±0.30	3.26±0.10^{+}	988.12±23	53.07±15.00^{+}	ENS
Dissotis princeps (Kunth.) Triana	Leaves & stems	96679	>200	>200	>200	>200	2.94±0.01^{+}	502.00±25.00	69.34±18.00^{+}	25.80±3.00
Erythrophleum lasianthum Corbishley	Leaves	120019	>200	147.00±3.00	58.60±2.00	>200	5.25±0.08^{+}	>1000	75.01±8.00^{+}	ENS
Euclea divinorum Hiern	Leaves	120020	107.00±2.00	66.60±0.60	133.00±2.00	177.00±1.00	8.30±0.02	>1000	71.12±8.00^{+}	ENS
Gomphocarpus fruticosus (L.) W.T. Aiton	Leaves	MN 1	51.04±3.00^{+}	>200	>200	>200	32.50±1.20	>1000	41.32±3.00	-
Harpephyllum caffrum Bernh.	Leaves	120021	62.50±5.00	68.30±0.90	106.00±4.00	135.00±0.10	2.41±0.01^{+}	248.00±16^{+}	77.47±7.00^{+}	6.40±1.60^{+}

Helichrysum kraussii Sch. Bip	Flowers, leaves &stems	96694	34.90±1.00^{+}	86.00±4.00	142.00±13.00	151.00±3.00	4.66±0.05^{+}	>1000	57.15±8.00^{+}	-
Leucas martinicensis (Jacq.) R.Br	Seeds & leaves	96690	121.00±0.40	100.00±0.10	>200	>200	34.50±0.20	>1000	72.62±12.00^{+}	16.03±8.00
Syzygium jambos (L.) Alston.	Leaves	119053	56.20±3.00	54.70±0.60	198.00±3.00	>200	1.17±0.30^{+}	>1000	86.06±5.00^{+}	3.79±0.90^{+}
Tabernaemontana elegans Stapf.	Leaves & stems	96692	192.00±0.60	66.70±2.00	>200	>200	157.00±8.00	>1000	63.09±9.00^{+}	ENS
Warburgia salutaris (G. Bertol.) Chiov.	Leaves	120013	>200	171.00±7.00	>200	>200	464.00±0.01	>1000	67.30±20.80^{+}	ENS
Actinomycin D[e]	-	-	(5.00±1.00) x10^{-3}	(3.90±0.10) x10^{-2}	(3.50±0.10) x10^{-2}	(3.00±1.00) x10^{-3}	-	-	-	-
Ascorbic acid[f]	-	-	-	-	-	-	1.98±0.01^{+}	285.90±26^{+}	-	-
Ipubrofen[g]	-	-	-	-	-	-	-	-	90.17±3.12^{+}	1.40±0.62^{+}

[a] Fifty percent inhibitory concentration; [b]2, 2-diphenyl-1-picrylhydrazyl radical; [c] Nitric oxide; [d] Cyclooxygenase-2; [e] Cytotoxicity positive control; [f] Antioxidant positive control; [g] Cyclooygenase-2 positive control; [-]Not tested; ENS – Extract not in sufficient amount for testing.

Results are reported as mean ± SD (n = 3). Statistical analysis was done using one-way ANOVA followed by Dunnett's Multiple Comparison Test using GraphPad Prism statistical software. Cancer cell cytotoxicity IC_{50} values statistically similar to *H. kraussii* on HeLa cells (IC_{50}: 34.90±1.00 µg/ml) were identified positive (+). The IC_{50} values statistically similar to the positive controls used in the DPPH, NO and COX-2 assay were identified positive (+) and IC_{50} values with statistically higher activity than the positive controls were identified *$p<0.01$

shade dried for two weeks and then ground to a fine powder. The dried powder (300 g) was macerated in distilled ethanol (2.5 L) and shaken for 48 h and thereafter filtered through Whatman No.1 filter paper using a Buchner funnel. The filtrate of each plant was collected and subjected to reduced pressure using a rotary evaporator (Büchi R-200) at 37-40 ºC. The extracts were kept in a cold room at -20 ºC until further use.

2.2.2 Cell culture

The human cervical epithelial carcinoma (HeLa), African green monkey kidney cells (Vero) and human embryonic kidney (HEK-293) cell lines were maintained in culture flasks which contained Eagle's Minimum Essential Medium (EMEM), whereas the human epidermoid carcinoma (A431) and human malignant melanoma (A375) cells were maintained in Dulbecco's Modified Eagles Medium (DMEM). All cell lines were supplemented with 1 % antibiotics (100 U/ml penicillin, 100 µg/ml streptomycin and 250 µg/L fungizone) and 10 % heat-inactivated foetal bovine serum. The cells were grown at 37 °C in a humidified incubator set at 5 % CO_2. Cells were sub-cultured after an 80 % confluent monolayer had formed.

2.2.3 In vitro cytotoxicity assay

Cytotoxicity was measured by the 2,3-Bis-(2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxyanilide salt (XTT) method using the Cell Proliferation Kit II. The method described by Berrington and Lall (2012) was used to perform the assay. Briefly, 100 µl cells were seeded in 96-well plates (1×10^5 cells/ml or 10,000 cells/well) and incubated for 24 h at 37 ºC in 5 % CO_2 for cell adherence. Stock concentrations of the extracts were prepared at 20 mg/ml in dimethylsulfoxide (DMSO) and further diluted in media to

appropriate concentrations. Cells were treated with 100 µl of the plant extracts at final concentrations ranging from 1.56-200 µg/ml for 72 h. Controls included a 2 % DMSO vehicle control and the concentration of the positive control, 'Actinomycin D' (stock concentration of 1 mg/ml in distilled water) ranged between 3.9×10^{-4}-0.05 µg/ml. After treatment, XTT (50 µl) was added to a final concentration of 0.3 mg/ml for 2 h. Blank plates were included which were prepared in the same manner above, however did not contain cells. The blank plates were used to compensate for the colour of the plant extract so as not to interfere with the absorbance of XTT. Absorbance was measured at 490 nm and 690 nm (reference wavelength) using a BIO-TEK Power-Wave XS multi-well plate reader (A.D.P, Weltevreden Park, South Africa). Each sample's concentration was tested in triplicate to calculate a fifty percent inhibitory concentration (IC_{50}) of cell viability.

2.2.4 Antioxidant assays

2.2.4.1 DPPH radical scavenging activity

The method as described Berrington and Lall (2012) was followed to determine the radical scavenging capacity (RSC) of the extracts. The stock solution of the positive control, ascorbic acid, and extracts were prepared at 2 mg/ml and 10 mg/ml in ethanol respectively. Twenty microlitres of the samples were added to the top wells of a 96-well plate and serially diluted to final concentrations ranging from 3.90-500 µg/ml and from 0.78-100 µg/ml for the extracts and ascorbic acid respectively. Ethanol at 10 % was used as the blank. Extracts which showed high inhibition at the lowest concentration tested (3.90 µg/ml) were tested at concentrations ranging from 0.78-100 µg/ml. Ninety microlitres of the ethanolic 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) solution (0.04 M) was added to each well and incubated for 30 min covered in foil. Negative colour controls were also prepared in the same

manner as above, however distilled water was added instead of DPPH. Absorbencies were measured at 515 nm using a BIO-TEK Power-Wave XS multi-plate reader using KC junior software. The IC_{50} values were calculated and the ascorbic acid equivalents were calculated as follows: (IC_{50} of extract X 200 mg ascorbic acid)/ IC_{50} of ascorbic acid. Each sample's concentration was tested in triplicate.

2.2.4.2 NO radical scavenging activity

The method as described by Mayur et al. (2010) was followed to determine the nitric oxide scavenging capacity of the extracts. The stock concentrations of the positive control, ascorbic acid, and extracts were prepared at 10 mg/ml in ethanol. Twenty microlitres of the extract's and ascorbic acid were added to the top well of a 96-well plate and serially diluted to final concentrations ranging from 7.81-1000 µg/ml. Ethanol at 10 % was used as the blank. Fifty microlitres of sodium nitroprusside (10mM) was added to all the wells and incubated at room temperature for 90 min. Thereafter, 100 µl Griess reagent was added to all the wells except for the negative colour control wells, where distilled water was added. Absorbencies were read at 546 nm using a BIO-TEK Power-Wave XS multi-well reader using KC Junior software and the IC_{50} value was calculated. Each sample's concentration was tested in triplicate.

2.2.5 Cyclooxygenase-2 assay

The method was performed as described by Reininger and Bauer (2006) to determine whether the sample's were able to inhibit human recombinant cyclooxygenase-2 (COX-2). To each well of a 96-well plate, 5 µl of the COX-2 enzyme (0.5 units/ well) was added to 180 µl of 100 mM TRIS buffer (pH 8.0) containing 5 µM porcine hematin, 18 mM L-

epinephrine, and 50 µM Na_2EDTA as co-factors. Stock concentrations of the extracts were prepared at 10 mg/ml in DMSO. Ten microlitres of the extracts were added to the wells at a final concentration of 10 µg/ml. Ibuprofen was used as the positive control and tested at final concentrations of 10 µM (stock concentration), 2 µM and 0.4 µM. DMSO at 5 % was used as the vehicle control. The reaction was initiated after 5 min incubation by adding 5 µl of arachidonic acid (10µM). The extracts, which showed >65 % inhibition at 10 µg/ml, were re-tested at concentrations ranging from 2.50-160 µg/ml to determine their IC_{50} values. The plates were incubated at room temperature for a further 20 min. Finally 10 µl of 10 % formic acid was added to stop the reaction. Samples were diluted in a ratio of 1:15 using assay buffer. Quantification of PGE_2, which is the main product of the reaction, was determined using the PGE_2 ELISA kit and the absorbance was measured at 405 nm using a BIO-TEK Power-Wave XS multi-well plate reader (A.D.P, Weltevreden Park, South Africa). The IC_{50} values of the four extract were calculated using Microsoft Excel.

2.2.6 Virus culture

The HSV-1 was obtained from the Christian Medical College and Hospital, Vellore, Tamil Nadu, India. A stock suspension of HSV-1 in Vero cells with titres of 1×10^{-7} $TCID_{50}$/ml (50% tissue culture infective dose) was prepared. The virus was diluted in serum-free media (EMEM) and used at final concentrations of 10 and 100 $TCID_{50}$ respectively.

2.2.7 In vitro anti- Herpes Simplex Virus type-1 assay

To determine the antiviral activity of the extracts, the cytopathic effect (CPE) inhibition assay against different virus challenge doses of 10 and 100 $TCID_{50}$ was used as described by Hu and Hsiung (1989). Briefly, 100 µl of Vero cells were seeded in a 96-well plate at

1×10^4 cells/well and incubated for 24 h at 37 ºC and 5 % CO_2 for cell adherence. Thereafter, plates were rinsed with serum-free media and treated with 100 µl different virus challenge doses (10 and 100 $TCID_{50}$). The plates were incubated for a further 1 h to allow virus absorption into the cells. The Vero cells act as the host-cell system in which the virus replication can be measured. After absorption, any unbound virus was removed by rinsing the plates with serum-free media. Stock concentrations of the extracts were prepared at 10 mg/ml in DMSO and further diluted to the final concentrations in media. The cultures were treated with 200 µl of plant extract with final concentrations ranging 12.50-100 µg/ml and incubated for a further 5 days. Each sample's concentration was performed in quadruplicate. Controls included the positive control Acyclovir at 5 µg/ml (100 % viral inhibition); a virus control where cells were infected with virus only (100 % virus growth); and a cell control, where cells were maintained in media only (100 % cell growth). Cells were observed every 24 h, using light microscopy, to examine the appearance of any cytopathic effects. Anti-HSV-1 activity was determined by the inhibition of cytopathic effect and compared with the 100% cell growth control and visually determined, *i.e.*, the protection offered by the extract to the Vero cells against the virus.

2.2.8 Statistical analysis

All results are reported as mean ± SD (n = 3). Statistical analysis was done using one way analysis of variance (ANOVA) followed by Dunnett's Multiple Comparison Test using the GraphPad Prism statistical software. For cancer cell cytotoxicity, extract's IC_{50} values were compared to that of *H. kraussii* on HeLa cells (34.90±1.00 µg/ml). This extract was used for comparison as it showed similar activity to the guidelines set by the American Cancer Institute, which sets the limit of activity for an extract at an IC_{50}< 30.00 µg/ml after 72 h exposure (Steenkamp & Gouws, 2006). Extracts statistically similar to *H. kraussii* were

identified with (+) and therefore had good activity. In the DPPH, NO and COX-2 assays, extracts which were statistically similar in activity to the positive controls were identified (+). Extracts which had statistically higher activity than the positive controls, were reported as *($p<0.01$).

3. Results and discussion

3.1 In vitro cytotoxicity

The sixteen ethanolic extracts were tested for cytotoxicity against cancerous A431, A375 and HeLa cells as well as the non-cancerous HEK-293 cell line. All the extracts showed IC_{50} values > 100 µg/ml against the non-cancerous HEK-293 cell line. *A. mellifera*, *G. fruticosus* and *H. kraussii* showed activity against the HeLa cell line with IC_{50} values < 60.00 µg/ml. *S.* jambos, however showed activity with IC_{50} values < 60.00 µg/ml against both the HeLa and A431 cell line (Table 1).

These plants and/ or compounds isolated from these plants have been previously reported by other researchers for their anticancer activity. According to previous reports, compounds 28-hydroxy-lup-20-(29)-en-30-al and 28-hydroxy-3-oxo-lup-20-(29)-en-30-al were isolated from *A. mellifera*, and showed good activity against non-small-cell broncho-pulmonary (NSCLC-N6) cells with IC_{50} values of 11.00 ± 0.02 and 15.00 ± 0.06 µg/ml respectively (Mutai et al. 2004). In a study by Fouche et al. (2008), a methanolic leaf/stem extract of *G. fruticosus* showed total growth inhibition values of 14.78 and 12.68 µg/ml on breast cancer (MCF-7) and melanoma (UACC62) respectively. A 1:1 chloroform: methanol leaf/stem extract previously showed toxicity on MCF-7, glioblastoma (SF-68) and kidney epithelial cells (Graham) with IC_{50} values of 9.00 µg/ml, 45.20 µg/ml, 28.60 µg/ml respectively (Lourens et al. 2011). *S. jambos* leaves were previously extracted with hexane and methanol and showed IC_{50} values of 150.00 and 600.00 µg/ml respectively on Vero cells (Sirivan, 2008). *H.*

kraussii has not previously been described for its cytotoxicity against cancer cells. These results are comparable to the results obtained by the above mentioned plants in the present study.

The statistical significance of the plant extracts IC_{50} values were compared to the IC_{50} (34.90±1.00 µg/ml) of *H. kraussiii*. The cytotoxicity of *H. kraussii* against HeLa cells was similar to the guidelines set by the American Cancer Institute, which sets the limit for activity of an extract at an IC_{50}< 30.00 µg/ml after 72 h exposure (Steenkamp & Gouws, 2006). *G. fruticosus* against HeLa cells, with an IC_{50} of 51.40±3.00 µg/ml, was found to be statistically similar to *H. kraussii*. Although this was the only extract statistically similar to *H. kraussii*, the IC_{50} values of *A. caffra* against HeLa cells and *S. jambos* against HeLa and A431 cells, were close to that of *G. fruticosus* against HeLa cells. These four extracts could therefore, be considered to screen for activity against other cancerous cell lines.

3.2 Antioxidant activity

The radical scavenging capacity of the plant extracts was determined using the DPPH and NO free radical scavenging assays. Free radicals are a major cause of DNA damage which results in the initiation of carcinogenesis. Therefore, the inhibition of free radicals could potentially aid in preventing carcinogenesis. Eight of the tested extracts showed good antioxidant activity (IC_{50} < 10.00 µg/ml). Four extracts showed IC_{50} values below 40.00 µg/ml and the remaining extracts showed low inhibition (IC_{50} > 70.00 µg/ml) (Table 1).

The highest DPPH scavenging activity was observed for *S. jambos* with an IC_{50} of 1.17±0.30µg/ml, which had statistically similar activity to that of ascorbic acid with an IC_{50} of 1.98±0.01 µg/ml. Earlier reports by researchers document the isolation of myricetin, myricitrin and gallic acid from the ethanolic leaf extract of *S. jambos*, which could contribute

towards the high antioxidant activity of the extract (Jayprakasham, 2010). In a previous study by Islam et al (2012), the ethanolic leaf extract of *S. jambos* showed less DPPH inhibition with an IC_{50} value of 14.10 µg/ml. In the NO scavenging assay, twelve extracts showed low activity with inhibition starting from 1000 µg/ml. Three extracts showed inhibition < 500 µg/ml, with the highest activity noted for *A. unedo* with an IC_{50} of 85.90±10 µg/ml which has statistically higher (*P<0.01) activity than that of ascorbic acid with an IC_{50} of 285.90±26 µg/ml. *A. unedo*, with an IC_{50} value of 4.51±0.20 against DPPH, also had statistically similar activity to ascorbic acid.

3.3 Cyclooxygenase-2 activity

The extract's activity against COX-2 was tested as it has been implicated in a number of cancer types. It has also been linked to increased cancer cell proliferation (Sobolewski et al. 2010). Therefore, a decrease in COX-2 activity could potentially inhibit the proliferation of cancer cells. The COX-2 inhibitory activity was tested for all the plant extracts. The plant extracts which showed high inhibition of the COX-2 enzyme at a concentration of 10.00 µg/ml were further selected to determine their IC_{50} values. Four extracts were selected to determine their IC_{50} values namely; *D. princeps*, *H. caffrum*, *L. martinicensis* and *S. jambos* (Table 1). *S. jambos*, with an IC_{50} of 3.79±0.90 µg/ml, showed the highest inhibition of COX-2, which was statistically similar to that of the ibuprofen, which showed an IC_{50} value of 1.40±0.62 µg/ml. *D. princeps* showed an IC_{50} values of 25.80±3.00 µg/ml, however was not statistically similar to ibuprofen. In a previous study a *D. princeps* ethanolic extract showed an inhibition of 22.10 % at a concentration of 250 µg/ml (Fawole et al. 2009). *H. caffrum* has also previously been tested for COX-2 inhibition and it was found that the non-polar extracts were more active than the polar extracts (Moyo et al. 2011). In this study however, the polar ethanolic extract of *H. caffrum* showed an IC_{50} of 6.40±1.60 µg/ml,

which was statistically similar to ibuprofen. *L. martinicensis* showed a high COX-2 inhibition of 72.62±12 % at 10 µg/ml, however when the IC_{50} (16.03±80 µg/ml) was calculated it was not found to be statistically similar to ibuprofen. This is the first report of the COX-2 inhibitory activity of *L. martinicensis* and *S. jambos*.

3.4 In vitro anti- Herpes Simplex Virus type-1 assay

Due to the anticancer activity of *G. fruticosus, H. kraussii* and *S. jambos* on cervical cancer cells, these extracts were further tested for antiviral activity (Table 1). Herpes simplex virus type-2 (HSV-2) is generally considered to be the causative agent for the development of genital herpes and therefore can cause cervical cancer. However, HSV-1, which is highly contagious, can also cause genital herpes, which causes genital or anal blisters or ulcers (WHO, 2017). Recent studies have suggested that the incidence of genital infections by HSV-1 has increased (Pereira et al. 2012). Therefore, the inhibitory activity of the plant extracts against HSV-1 was determined.

S. jambos, at 50.00 µg/ml exhibited potential anti-viral activity with 100 % viral inhibition when tested at the highest viral dose ($100TCID_{50}$). At a viral dose of 10TCID50, *S. jambos* was able to inhibit 100 % of the virus at all four concentrations tested (Table 2). This was comparable to the positive control, acyclovir, which showed 100 % inhibition at 1.00 µg/ml. *H. kraussii* showed similar activity to *S. jambos,* where 100% viral inhibition was observed at all the concentrations tested when the viral dose was $10TCID_{50}$ and therefore was also comparable to the positive control, acyclovir. At a viral dose of $100TCID_{50}$, *H. kraussii* inhibited 100% of the virus at the highest concentrations tested (100 µg/ml). *G. fruticosus,* however exhibited no antiviral activity at the tested concentrations at both $10TCID_{50}$ and $100TCID_{50}$ viral challenge doses.

In a study by Sirivan et al. (2008) a plaque reduction assay was performed using different extracts of *S. jambos* against HSV-1 and HSV-2. The extracts were each tested at a concentration of 100 µg/ml and were found to inhibit both HSV-1 and HSV-2 by more than 50 % when using the hexane and dichloromethane leaf extracts, whereas the methanol leaf extract showed 11.20 and 30.60 % inhibition against HSV-1 and HSV-2 respectively.

Table 2

The anti-HSV-1 (IC_{50} in µg/ml) activity of the selected plant extracts

Plant extract	IC_{50}[a] (µg/ml) Vero	Concentration tested (µg/ml)	% viral inhibition	
			$10TCID_{50}$	$100TCID_{50}$
Gomphocarpus fruticosus	52.97	100	0% at all concentration	0% at all concentration
		50		
		25		
		12.5		
Helichrysum kraussii	264.01	100	100% at all concentration	100%
		50		75%
		25		0%
		12.5		0%
Syzygium jambos	218.19	100	100% at all concentration	100%
		50		100%
		25		50%
		12.5		0%
Cell control (without virus and extract)	-	-	100%	100%
Virus control	-	-	0%	0%
Acyclovir[b]	-	5	100%	100%

[a] Fifty percent inhibitory concentration; [b] Anti-viral positive control

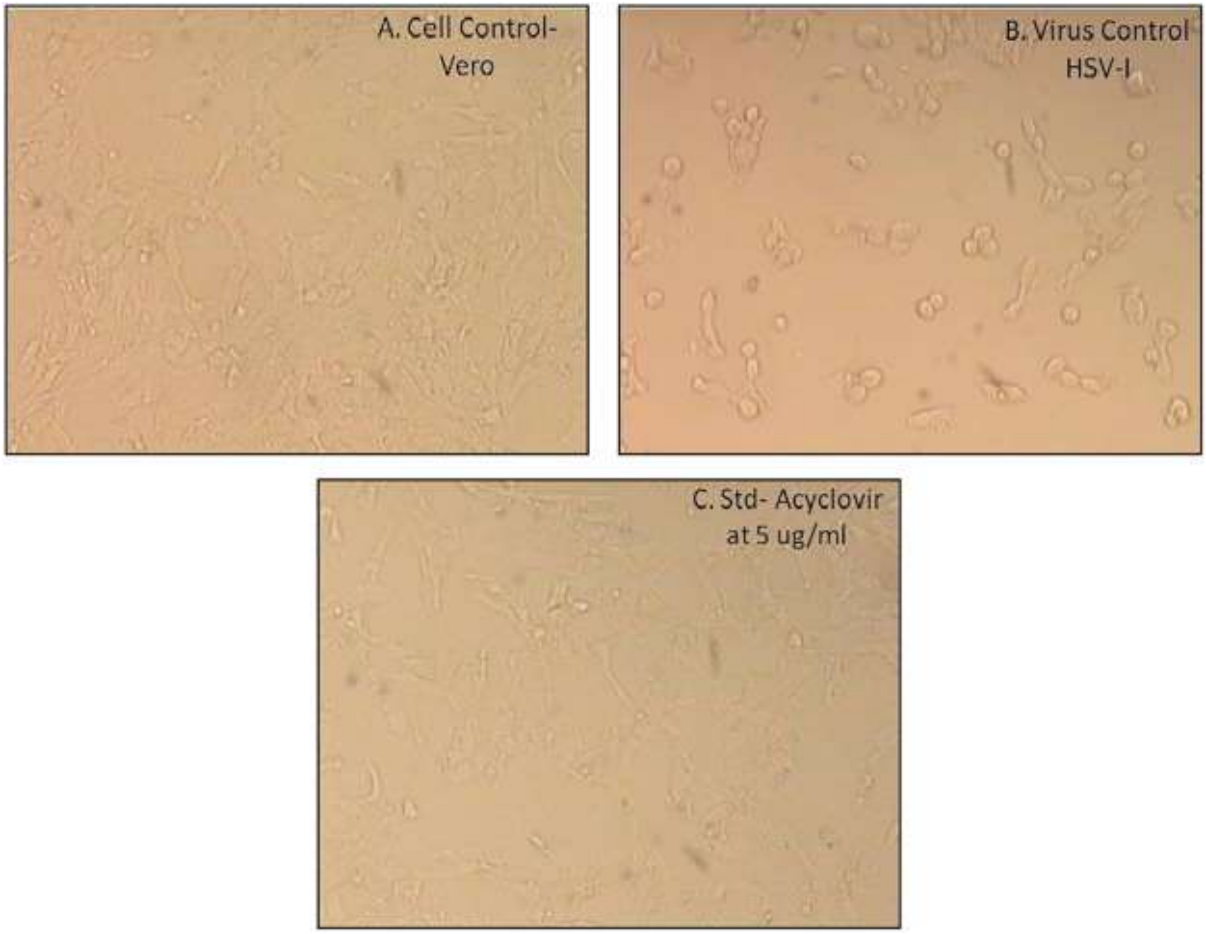

Fig. 1.

Light microscopy (20 × magnification) of A) Vero cells where 100% cell growth is observed, B) Vero cells are infected with the HSV-1 (0% cell growth) and C) HSV-1 infected Vero cells treated with 5 µg/ml anti-viral positive control, acyclovir where there is 100% cell growth.

4. Conclusion

In this study four extracts exhibited anti-cancer activity against the HeLa and A431 cell lines. Three of these extracts were further tested for anti-viral activity against HSV-1 and the highest activity was observed by *S. jambos* which showed 100 % viral inhibition at 50.00 µg/ml at the highest viral challenge dose. *S. jambos* also showed the greatest COX-2 and DPPH inhibitory activity. This is the first report of the activity of the selected plants for their cytotoxicity on the selected cell lines. The collective data for the anticancer, antioxidant and anti-inflammatory activity of *S. jambos* shows the potential of the plant extract to be considered for its application as a chemopreventive and anticancer agent. Furthermore, *S.*

jambos could be considered a promising extract for anti-viral activity. This study further shows the potential of *H. caffrum* as an anti-inflammatory agent and the opportunity to test *A. mellifera, G. fruticosus, H. kraussii* and *S. jambos* to be tested against other cancerous cell lines.

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Conflict of interest

The authors declare that there are not conflicts of interest.

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