The antiviral activity of compounds isolated from Kenyan Carissa edulis (Forssk.) Vahl

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Citation

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Abstract

Extracts of a Kenyan medicinal plant, Carissa edulis (Forssk.) Vahl, were subjected to bioactivity guided phytochemical analysis. Pure compounds; lupeol, oleuropein, carissol and -amyrin were subsequently isolated. In vitro evaluations of the compounds against sensitive and resistant viral strains of Herpes simples virus types 1 revealed a significant antiviral activity for lupeol (EC_{50} 2.98-4.2µg/ml) with a selectivity index >38 (CC_{50} >166.0µg/ml). At a concentration of 10.0µg/ml, the compound reduced the viral yields in Vero E6 cells by 98.3%. On oral administration to mice at 20.0µg/ml following a cutaneous viral infection, a delayed onset of infections of slow progression to mild and sever zosteriform lesions were observed (p £ 0.05 test Vs control by repeated measures ANOVA). The mice receiving treatment with the compound also exhibited increased mean survival times as opposed to control (p ≤ 0.05 test verses control by Student's t-test) with a therapeutic index >5 (LD_{50} >100.0µg/ml). The results indicate promising antiviral activity of lupeol and necessitate further examinations of efficacy and safety in higher mammals.

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INTRODUCTION

Herpes simplex virus (HSV) infections are among the most common diseases of humans [1,20] . In 1999, the estimate of the number of sufferers to these infections worldwide was approximated as 86 million people [7] and since then, the prevalence of HSV infection has been increasing [20] . In sub-Saharan Africa high seroprevalence rates of between 60 to 80 % in young adults have been recorded in population based studies [6,16,23] .

The most widely used drug for prophylaxis and treatment of HSV infections is acyclovir [10] . However, the long-term therapy necessary for management of infection has been associated with development of clinically resistant strains of the virus [15,18] . There is therefore a need to identify new agents for management of HSV infections.

Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well-being. Artemisinin (Quinghaosu) from Artemisia annua is such one example [8] . In the quest for new remedies to treat old and emergent diseases, attention is therefore being given to discovering the active ingredients from plants that have been used routinely and traditionally for disease alleviation. The Kenyan Carissa edulis is one such plant. Extracts of the plant had previously shown significant in vitro and in vivo antiviral activity for HSV types 1 and 2 [22] . This report is a follow up of the extracts and results are presented of the antiviral activity of the pure compounds isolated from them.

MATERIALS AND METHODS

PLANT COLLECTION

The Carissa edulis medicinal plant was collected from its natural habitat in Gitoro forest of Meru, Eastern Province, Kenya. The roots were collected with acceptable bio-conservation methods and a voucher specimen (Mungai, Rukunga and Tolo; No. 076) is on deposit at the East African Herbarium, National Museums of Kenya, Nairobi, Kenya.

EXTRACTION AND ACTIVITY GUIDED PHYTOCHEMICAL ANALYSIS

Extraction and activity guided phytochemical analysis was carried out concurrently. Briefly; 150 g of powdered root bark was extracted successively using diethylether and methanol and the extracts dried in vacuo. The yields for the diethylether extract was loaded into a column packed with silica gel and eluted gradient wise with increasing

concentrations of ethylacetate in hexane. The fractions of 10 ml each were collected and monitored on pre-coated thin layer chromatography (TLC) plates. Similar fractions were pooled according to the TLC characteristics. One such pooled fraction, coded CEE-3, had reasonable antiviral activity in vitro than the others and was a mixture of three compounds. Further purification of this fraction was carried out using column chromatography packed with reverse phase (RP)-C18 Silica gel and eluted with increasing concentrations of acetonitrile in methanol, starting from 5 to 10 % acetonitrile. Fractions were monitored on RP-C18 TLC analytical plates and fractions pooled as appropriate. Three pure compounds coded; CEE-3A, CEE-3B and CEE-3C were subsequently obtained.

The methanol extract was loaded into a column packed with sephadex LH-20 and eluted isocratically using methanol (MeOH). Ten milliliter (10 ml) fractions were obtained and monitored through TLC. Following bioactivity, one of the fractions was purified using a column packed with RP-C18 and eluted with MeOH:H_2O (35:65). One pure compound coded as CEM-1E was isolated.

These four compounds; CEE-3A, CEE-3B, CEE-3C and CEM-1E were identified by comparison of their spectroscopic profiles with published data and found to be lupeol [17] , -amyrin [24] , carissol [24] and oleuropein [4,14] respectively.

VIRUSES AND CELLS

The Vero E6 cells were donated by the Virology department, Toyama Medical and Pharmaceutical University, Toyama, Japan. The cells were grown in 5% CO_2 at 37 ^{o} C in MEM supplemented with 5% FBS for cell growth, and 2% FBS for cell maintenance.

The herpes simplex virus (HSV) strains used in the study were wild-type 7401H HSV-1 [11] , thymidine kinase deficient (TK–) B2006 HSV-1 [5] and acyclovir resistant (AP^{r}) 7401H HSV-1 [13] . The viruses were donated by the Virology Department, Toyama Medical and Pharmaceutical University, Toyama, Japan. The virus stocks were prepared from infected Vero E6 cells. The infected cultures were frozen and thawed three times to lyse the cells, and centrifuged at 3000 rpm for 15 min. Their supernatants, containing HSV, were harvested and stored at –80 ^{o} C until use.

PLAQUE INHIBITION ASSAY

The compounds were examined for extent of inhibition of plaques on HSV infected cells as a measure of anti-viral activity in vitro using the method described by Kurokawa et al. [11,12] . Briefly, Vero E6 cells were cultured to a confluent monolayer in MEM supplemented with 5% FBS in 5% CO_2 at 37 ^{o} C. The cells were infected with 100 plaque forming units (PFU) of HSV and left to adsorb for 1 h at room temperature. The cells were then overlaid with MEM supplemented with 2% FBS and 0.8% Methylcellulose (MC) containing various concentrations of the compounds and incubated at 37 ^{o} C in 5% CO_2 for 2 days. The infected treated cells were fixed in formalin, washed and stained with 0.03% methylene blue solution. The plaques, appearing as transparent dots against a blue background, were counted using a dissecting microscope and the percent plaque inhibition calculated against control. The effective concentration inhibiting formation of plaques by 50% (EC_{50}) was determined from two independent experiments.

CELL CYTOTOXICITY ASSAY

The cytotoxic concentration causing 50% cell lysis and death (CC_{50}) was determined for the compounds by the method described elsewhere [22] . Briefly, Vero E6 cells were seeded at a concentration of 2.5×10^{4} cells/well in 24-well plates and grown in 5% CO_2 at 37 ^{o} C for 2 days. The culture medium was replaced by fresh medium containing compounds at various concentrations, and cells further grown for 24 h. The cells were treated with trypsin and the number of viable cells determined by the trypan blue exclusion method. The concentration of compound reducing cell viability by 50% (CC_{50}) was determined from a curve relating percent cell viability to the concentration of compounds.

VIRUS YIELD REDUCTION ASSAY

Lupeol was compared for its anti-viral activity with acyclovir on the growth of the wild type strain of HSV-1 in the virus yield reduction assay as described elsewhere [22] . Briefly, confluent monolayers of Vero E6 cells were infected with HSV-1 at multiplicity of infection of 5 (5 M.O.I). Separate dishes each containing infected cells were treated with lupeol at various concentrations (0-10 µg/ml). The cells were incubated in 5% CO_2 at 37 ^{o} C for 24 h and lysed. The lysed cultures were collected in 15 ml tubes and centrifuged at 3000 rpm for 10 min and supernatant harvested. A serial dilution of each supernatant was prepared in MEM and the virus titre determined by the plaque inhibition assay.

DETERMINATION OF EFFICACY OF LUPEOL IN MICE

Balb/C mice, reared at the Kenya Medical Research

Institute's (KEMRI) animal facility, were used in the experiments. The animals were handled according to the guidelines laid down by the Animal Care and Use Committee of the KEMRI. The in vivo evaluation of the efficacy of lupeol was carried out using the method described by Kurokawa et al. [12] . Briefly, female Balb/C mice of 7 weeks old and weighing approximately 20 g were acclimatized for one week in the experimental room. The animals were fed with food (Mice cubes, Unga feeds, Kenya) and water ad libitum. The mid flank of each mouse was shaved using an electric hair trimmer (Wahl super taper, England) and hair completely removed by applying a chemical hair remover (Shiseido, Co., Ltd., Tokyo, Japan) on the shaved area. The mice were randomly divided into treatment groups each comprising of 5 mice. The shaved mid flank of each mouse was scratched using a bundle of G27 needles and the scarified area infected with 1×10^{6} PFU HSV-1. The groups were administered with the test drug or acyclovir or placebo orally three times a day for seven consecutive days. The development of skin lesions and mortality were continuously monitored every 8 h daily and scored as follows: 0-no lesion; 2-vesicles in local region; 4-erosion and/or ulceration in the local region; 6-mild zosteriform; 8-moderate zosteriform and 10-severe zosteriform and death. The mice were fed and observed for 30 days before the experiment was terminated.

DATA ANALYSIS

In the in vitro assays, the mean number of plaques was used to calculate the percentage of plaques inhibited. A curve relating percent plaque inhibition against concentration (a dose response curve) was used to calculate the inhibitory concentration for 50% (EC_{50}). In the cytotoxicity experiments, the number of viable cells in the untreated control was used to calculate the percent of cells lysed in the treated cells. The cell cytotoxic concentration (CC_{50}) was determined from a plot relating percent cell viability against each tested concentration.

In the in vivo experiments, the Student's t-test was used to evaluate the significance of differences between control and compound treated mice in mean survival times and mean times at which skin lesions were initially scored 2 (vesicles in local region) or 6 (zosteriform lesions) after infection. The repeated measure analysis of variance (ANOVA) with Dunn's procedure as a multiple comparison procedure was used to analyze the interactions between treatment and progression of infection in mean lesions scores from day 3 to 10 following HSV-1 infection. A p-value of 0.05 was defined as statistically significant.

The 1998 StatView computer software by SAS Institute Inc. USA was used for the statistical analysis.

RESULTS

ANTIVIRAL ACTIVITY OF THE COMPOUNDS

The in vitro antiviral activities of the compounds against sensitive and resistant viral strains of HSV are presented as Table 1. Of the four compounds, lupeol exhibited the most potent antiviral activity; EC_{50} at 2.98 µg/ml for 7401H HSV-1, 3.66µg/ml for AP^{r}7401H HSV-1 and 4.2 µg/ml for the TK^{-}B2006 HSV-1. The EC_{50} concentrations were far below the toxic level (CC_{50} >160.0 µg/ml) giving lupeol a selectivity index of >38. The 7401H HSV-1 strain was sensitive to oleuropein and carissol; EC_{50} at 4.83 and 3.76 µg/ml respectively, however, no activity was registered for oleuropein in both the acyclovir resistant viral strains at 5 µg/ml. -amyrin did not have activity against the 7401H HSV-1 strain at the highest tested concentration of 5.0 µg/ml and was therefore not examined any further. Acyclovir, the reference drug, had activity for 7401H HSV-1; EC_{50} at 0.45 µg/ml, but did not show any activity for the other strains at the highest tested concentration of 10.0 µg/ml.

Figure 1

Table 1: antiviral activity of the compounds

Compound	EC_{50} (µg/ml)*			CC_{50}	S.I
	7401H HSV-1	AP^{r} 7401H HSV-1	TK^{-} B2006 HSV-1		
Lupeol	2.98	3.66	4.2	>160.0[a]	>38
Oleuropein	4.83	>5.0[a]	>5.0[a]	>160.0[a]	>32
Carissol	3.76	NE	NE	>10.0[a]	>2
β -amyrin	>5.0[a]	NE	NE	>10.0[a]	ND
Acyclovir	0.45 ± 0.14	>10.0[a]	>10.0[a]	>100.0	>200

The letters in parenthesis in the table represent the following; * = Mean of two independent experiments, [a] = Highest tested concentration. The abbreviations represent the following; **NE** = Not examined **ND** = Not determined

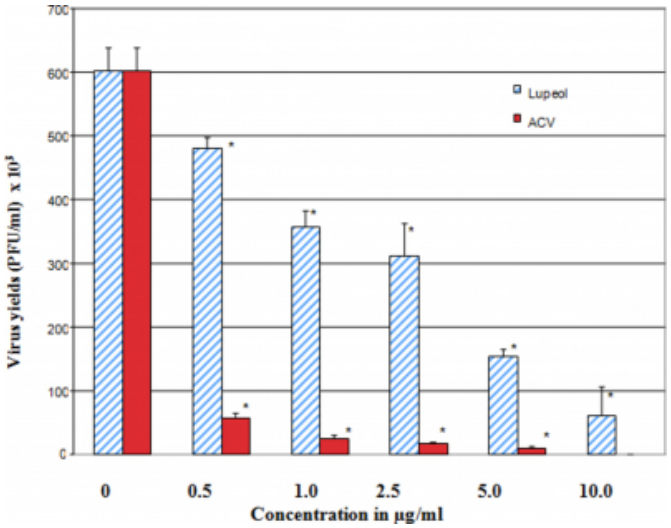
VIRUS YIELDS IN VERO E6 CELLS TREATED WITH LUPEOL OR ACYCLOVIR

The bar graph Figure 1 compares the virus yields of 7401H HSV-1 in Vero cells treated with varying concentrations of lupeol or acyclovir. Lupeol reduced the virus yields in a dose dependent manner, the highest concentration of 10.0 µg/ml reducing the yields to less than $1x10^{5}$ PFU/ml (~ 98.3% virus yield reduction). At 2.5 µg/ml, lupeol reduced the virus yields by half the amount registered in untreated cells. On the other hand, acyclovir reduced the viral yields more sharply, the 2.5 µg/ml concentration reducing the yields significantly (to less than $1x10^{2}$ PFU/ml) and completely at 10.0 µg/ml. The mean virus yields at each tested

concentration for both lupeol and acyclovir were statistically significant (p≤0.05 test verses control by Student's t-test).

Figure 2

Figure 1: The virus yields in 7401H HSV-1 infected Vero E6 cells treated with lupeol or acyclovir . The error bars indicate the levels of deviation within the mean of three independent experiments at each tested concentration. * = ? 0.05 (Test verses control by StudentÂ's t-test).



EFFICACY OF LUPEOL IN A CUTANEOUS HSV-1 INFECTION MODEL.

Table 2 presents the efficacy of lupeol on 7401H HSV-1 cutaneous infection in mice. Three doses (5, 10 and 20mg/kg) were orally administered to mice over a period of 7 days and observations for onset of infection, progression, mean survival times and mortality recorded. Vesicles in local region (score 2) were observed on the 4 th , 5 th and 6 th days for 5, 10 and 20 mg/kg treatments respectively. Vesicles appeared on controls by the 4 th day while the group on acyclovir at 5mg/kg on the 7 th day. Mild zosteriform lesions (score 6) were observed on 7 th day for groups receiving treatment at 5 and 10 mg/kg while for the 20 mg/kg and acyclovir groups, lesions were noted on 8 th day. The mean survival times for lupeol treatments at 5 and 10 mg/kg were similar to the control group (approximately 8 days) while the group on 20 mg/kg was 9 days (p0.05 test Vs control by Student's t-test). A high mortality was observed in all the groups that received lupeol treatment. No toxicity was observed in uninfected mice treated with the highest dose of lupeol (results not shown).

Figure 2 outlines the interaction line plots for progression of 7401H HSV-1 infection in mice following oral treatments with lupeol at 5, 10 and 20 mg/kg. The progression of infection in mice treated with 5 mg/kg was similar to that of control, where once the infection was on progresses, it continued uninterrupted until the final lethal stages. The 10 mg/ml treatment delayed the progression of infection in the treatment phase, however on withdrawal of treatment on the 7 th day, the infection progressed faster to the lethal stages. The mice on 20 mg/kg had a much slower progression of infection than the 10 mg/kg treatment. The influence of treatment on progression of infection at 10 and 20 mg/kg were statistically significant {p0.05 test Vs control by repeated measures ANOVA (Benferroni/Dunn)}. Acyclovir treatment contained progression of infection to below mild zosteriform lesions, with a statistical significance {p0.05 test Vs control by repeated measures ANOVA (Benferroni/Dunn)}.

Figure 3

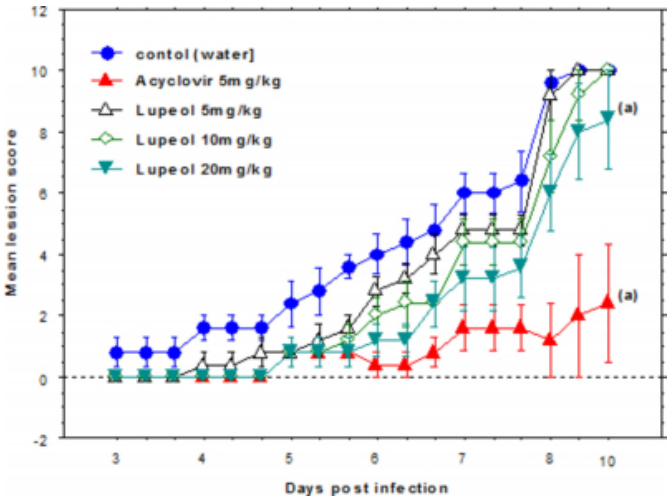
Table 2: The efficacy of lupeol in a cuteneous 7410H HSV-1 infection in mice

Animals	Treatment	Mean time (Days ± S.D)			Mortality[c]
	mg/kg	Score 2[a]	Score 6[a]	Survival[b]	
Control	0	4.2 ± 0.45	6.6 ± 0.89	8.0 ± 0.71	5/5
Acyclovir	5	7.0 ± 2.16	8.0 ± 0.0	9.0 ± 0.0	1/5
Lupeol	5	4.8 ± 0.84	7.4 ± 0.89	8.2 ± 0.45	5/5
Lupeol	10	5.2 ± 1.10	7.8 ± 0.84	8.8 ± 0.84	5/5
Lupeol	20	6.0 ± 1.23	8.0 ± 0.82*	9.5 ± 1.00*	4/5

The letters and symbols in parenthesis represent the following; * = $p \leq 0.05$ (Test Vs control by Student's t-test) [a] = Mean times at which score 2 or 6 was first observed, [b] = Surviving mice were not included for the calculation of mean survival times and [c] = Number of dead mice against total number surviving in the group. Mortality was calculated on day 10.

Figure 4

Figure 2: The interaction line plots for progression of 7401H HSV-1 infection in Balb/C mice following oral treatments with increasing doses of lupeol. In the figure, = ? 0.05 Test Vs control by repeated measures ANOVA (Benferroni/Dunn). Error bars are deviations within the mean of 5 mice in each group.



DISCUSSION

Three of the four compounds isolated from the root bark of C. edulis; lupeol, oleuropein and carissol demonstrated anti-HSV activity in vitro. However, it was only lupeol that had activity for both the sensitive and resistant viral strains. Lupeol, a triterpene, is found in various edible plants, fruits and medicinal plants used in many parts of the world [3,9,19] . The compound has been shown to exhibit strong anti-inflamatory, anti-arthritic, anti-mutagenic, anti-malarial and anti-viral activity in in vitro and in vivo systems [21,19] . The in vitro anti-HSV activity of lupeol has previously been demonstrated by other researchers. Tanaka et al., [21] , reported that lupeol isolated from the roots of Strobilanthes cusia BREMEK (Acanthaceae) had anti-HSV activity for the KOS strain HSV-1 (EC_{50}: 11.7µM) and showed 100% inhibition of virus plaque formation at 25µg/ml in Vero cells. The results of this study confirm these findings and move a step further by demonstrating the sensitivity of the acyclovir resistant strains to the compound. The virucidal effect of lupeol, as witnessed in the virus yield reduction assay, was another pointer of the antiviral potential of the compound. Since the acyclovir resistant strains were sensitive to lupeol, this indicated possibilities of a difference in the mechanism of antiviral action to that of acyclovir that needs to be explored. The cytotoxic level of lupeol in Vero cells was well above EC_{50} giving it a good selectivity index. A similar level of cytotoxicity (CC_{50}; 196µg/ml) has been reported by other researchers in Vero cells [2] .

The in vivo efficacy of lupeol for cutaneous HSV infection in mice was however weak. Even though the progression of infection was comparatively slow (against control), leading to improved mean survival times, there was high mortality in all the treatment groups. It is difficult to provide explanations for these observations at this stage other than being speculative. For instance, the observed low activity could be attributed to biological factors in play in an in vivo environment. Since efficacy was investigated following an oral route, there are possibilities that once in the alimentary canal, lupeol could have been bio-transformed into other inactive or less active forms by the digestive system and thereby reducing it potency. Alternatively, perhaps due to poor bioavailability, the drug was not readily absorbed from the alimentary canal so as to reach the target sites of infection and thus the low effect. However, as already mentioned, these are just assumptions since this line of thought needs to be verified with data. The LD_{50} for lupeol was well above the therapeutic level {>100 mg/kg (results not shown)} and therefore toxicity could not have contributed to the high mortality.

CONCLUSION

Lupeol is a promising candidate for development into an anti-HSV agent. As means of attaining this goal, it would be necessary to determine its mechanism of anti-HSV action since this would help explain the activity on the resistant viral strains. The bioavailability and pharmacokinetic profiles of the drug also needs to be examined to determine the best dosage regimen and administration route for further investigations. The results show a great potential and warrants further work, possibly in higher mammals.

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References

1. Ballard, R., (2001). The role of serological screening in the management of genital herpes. The International Congress of Sexually Transmitted Infections (ISSTDR/IUSTI), 24–27 June 2001, Berlin, Germany.
2. Badami, S., Vijayan, P., Mathew, N., Chandrashekhar, R., Godavarthi, A., Dhanaraj, S.A. and Suresh, B. (2003). In vitro Cytotoxic properties of Grewia tiliaefolia bark and Lupeol. India Journal of Pharmacology. 35: 250-251.

3. Beveridge, T.H., Li, T.S. and Drover, J.C. (2002). Phytosterol content in American ginseng seed oil. Journal of Agriculture and Food Chemistry. 50:744-750.
4. Calis, H., Hosny, M., Khalifa, T., Nishibe, S., (1993). Secoiridoids from Fraxinus angustifolia. Phytochemistry. 33 (6): 1453-1456.
5. Dubbs, D.R. and Kit, S. (1964). Mutant strains of herpes simplex virus deficient in thymidine kinase inducing activity. Virology. 22: 293-502.
6. Gwanzura, L., McFarland, W. and Alexander, D., (1998). Association between human immunodeficiency virus and herpes simplex virus type 2 seropositivity among male factory workers in Zimbabwe. The Journal Infectious Diseases. 177: 481–484.
7. Halioua B and Malkkin J E., (1999). Epidemiology of Gential Herpes -Recent Advances. European Journal of Dermatology. 9(3): 177-184.
8. Hoareau, L. and DaSilva, E.J, (1999). Medicinal plants: a re-emerging health aid. EJB Electronic Journal of Biotechnology. 2(2): 56-70.
9. Kakunda, R., Iijima, T., Yaoita, Y., Machida, K. and Kikuchi, M. (2002). Triterpenoids from Gentiana scabra. Phytochemistry. 59:791-794.
10. Kleymann, G., (2003). Novel agents and strategies to treat herpes simplex infections. Expert Opinion on Investigational Drugs. 12: 165–183.
11. Kurokawa, M., Hase, K., Xu, H., Yamamura, J., Koyasu, M., Sato, H., Kadota, S., Hozumi, T., Namba, T. and Shiraki, K., (1993). A novel procedure for the identification of a fraction with anti-herpes Simplex Virus type-1 actvity in vivo from hot-water extract of traditional medicines, Geum japonicum THUNB. Journal of Medical and Pharmaceutical Society for WAKAN-YAKU. 10:195-202.
12. Kurokawa, M., Hozumi, T., Tsurita, M., Kadota, S., Namba, T. and Shiraki K., (2001). Biological characterization of Eugeniin as an anti-Herpes Simplex Virus Type 1 compound in vitro and in vivo. The Journal of Pharmacology and Experimental Therapeutics. 297:372-379.
13. Kurokawa, M., Nagasaka, K., Hirabayashi, T., Uyama, S., Sato, H., Kageyama, T., Kadota, S., Ohyama, H., Hozumi, T., Namba, T. and Shiraki, K., (1995). Efficacy of traditional herbal medicines in comination with acyclovir against herpes simples virus type 1 in vitro and in vivo. Antiviral Research. 27: 19-37.
14. Kuwajima, H., Morita, M., Takaishi, K., Inoue, K., Fujita, T., He, Z., Yang, C., (1992). Secoiridoid, coumarin and secoiridoid-coumarin glucosides from Fraxinus chinensis. Phytochemistry, 31 (4), 1277-1280.
15. Morfin, F., Thouvenot, D., (2003). Herpes simplex virus resistance to antiviral drugs. Journal of Clinical Virology. 26: 29–37.
16. Obasi, A., Mosha, F., Quigley, M., Sekirassa, Z., Gibbs, t., Munguti,K., Todd, J., Grosskurth, H., Mayaud, P., Changalucha, J., Brown, D., Mabey, D. and Hayes R. (1999). Antibody to herpes simplex virus type 2 as a marker of sexual risk behaviour in rural Tanzania. The Journal of Infectious Diseases. 179: 16-24.
17. Pakrash, S.C., Datta, S., Ghoshi-Dastidar, P.P., (1968). Indian Medicinal Plants –XVII. Phytochemical examination of Carissa species. Phytochemistry. 7, 495-496.
18. Reyes, M., Shaik, N.S., Graber, J.M., Nisenbaum, R., Wetherall, N.T., Fukuda, K., Reeves, W.C., (2003). Acyclovir-resistant genital herpes among persons attending sexually transmitted disease and human immunodeficiency virus clinics. Archives of Internal Medicine. 163: 76–80.
19. Saleem, M., Kaury, S., Kweony, M-H., Adhami, V.M., Afaq, F. and Mukhtar, H., (2005). Lupeol, a fruit and vegetable based triterpene, induces apoptotic death of human pancreatic adenocarcinoma cells via inhibition of Ras signaling pathway. Carcinogenesis. 26 (11):1956–1964.
20. Smith, J.S. and Robinson, N.J., (2002). Age-Specific prevalence of infection with herpes simplex virus types 2 and 1: A global review. The Journal of Infectious Diseases. 186(Suppl 1): S3-S28.
21. Tanaka, T., Ikeda, T., Kaku, M., Zhu, X-H., Okawa, M., Yokomizo, K., Uyeda, M. and Nohara. T., (2004). A New Lignan Glycoside and Phenylethanoid Glycosides from Strobilanthes cusia BREMEK. Chemistry and Pharmaceutical Bulleletin. 52(10): 1242-1245.
22. Tolo, F.M., Rukunga, G.M., Muli, F.W., Njagi, E.N.M., Njue, W., Kumon, K., Mungai, G.M., Muthaura, C.N., Muli, J.M., Keter, L.K., Oishi, E., and Kofi-Tsekpo, M.W., (2006). Anti-viral activity of the extracts of a Kenyan medicinal plant Carissa edulis against herpes simplex virus. Journal of Ethnopharmacology. 104: 92-99.
23. Wagner, H.U., Van Dyck, E., Roggen, E., (1994). Seroprevalence and incidence of sexually transmitted diseases in rural Ugandan population. International Journal of STD and AIDS. 5: 332–337.
24. Zafar, N., Ataullah, M., Sirajuddin, N.S. (1985). Isolation of a new triterpenic alcohol from Carissa carandas. Pakistan Journal of Scientific and Industrial Research. 28(6), 378-8.

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