

Review

Antiviral agents from plants and herbs: a systematic review

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Background and aims: Many antiviral compounds presently in clinical use have a narrow spectrum of activity, limited therapeutic usefulness and variable toxicity. There is also an emerging problem of resistant viral strains. This study was undertaken to examine the published literature on herbs and plants with antiviral activity, their laboratory evaluation *in vitro* and *in vivo*, and evidence of human clinical efficacy.

Methods: Independent literature searches were performed on MEDLINE, EMBASE, CISCOP, AMED and Cochrane Library for information on plants and herbs with antiviral activity. There was no restriction on the language of publication. Data from clinical trials of single herb preparations used to treat uncomplicated viral infections were extracted in a standardized, predefined manner.

Results: Many hundreds of herbal preparations with antiviral activity were identified and the results of one

search presented as an example. Yet extracts from only 11 species met the inclusion criteria of this review and have been tested in clinical trials. They have been used in a total of 33 randomized, and a further eight non-randomized, clinical trials. Fourteen of these trials described the use of *Phyllanthus* spp. for treatment of hepatitis B, seven reporting positive and seven reporting negative results. The other 10 herbal medicines had each been tested in between one and nine clinical trials. Only four of these 26 trials reported no benefit from the herbal product.

Conclusions: Though most of the clinical trials located reported some benefits from use of antiviral herbal medicines, negative trials may not be published at all. There remains a need for larger, stringently designed, randomized clinical trials to provide conclusive evidence of their efficacy.

Introduction

Many hundreds of plants worldwide have a place in folk medicine as treatments for microbial infections and antimicrobial activity of extracts *in vitro* may be readily assessed in microbiology laboratories. While many so tested are reported to show inhibitory effects against a range of organisms, few proceed to animal testing and even fewer to clinical trials in humans. Laboratory testing can at best be only a very crude, though relatively inexpensive and rapid screen, while *in vivo* testing is very costly and time consuming.

Numerous effective antibacterial and antifungal drugs are already available but there are limited types of antiviral agents and increasing demand for them, particularly for treatment of hepatitis and human immunodeficiency virus (HIV).

This review was undertaken to examine the range of plants or herbs reported in the scientific literature that have been tested for antiviral properties in laboratories, animals and humans.

Methods

Computerized literature searches were performed on MEDLINE (via PubMed), EMBASE, CISCOP, AMED and Cochrane Library from their inception to November 2002. Primary search terms used were 'herb' OR 'plant' AND 'antiviral' AND 'clinical trials'. Further searches were undertaken using the names of individual plants with antimicrobial effects as documented *in vitro* and also individual viruses reported in clinical trials. Departmental files were searched and further papers located from bibliographies. No restriction on the language of publication was applied. Literature searches not restricted to clinical trials were also carried out to determine the scale of *in vitro* studies and one MEDLINE search on herpes simplex virus was analysed in detail as an example.

Clinical trials were included in the analysis if they reported experimental use of a single plant extract for treatment of uncomplicated viral infection in humans.

Studies using herbal mixtures or using herbal medicines solely for prevention of viral infections were excluded. *Echinaceae* spp., *Silybum marianum* and *Eleutherococcus senticosus* have been reported to have antiviral activity but were also excluded because their effects are believed to be predominantly immunomodulatory. Manufactured synthetic replicas of plant products are not herbal medicines and these were also excluded.

All data were extracted by the first author into predefined tables and validated by the second author. Methodological quality of all clinical trials was assessed according to the system developed by Jadad *et al.* (maximum score 5) [1].

Results

Herpes simplex search

One literature search was chosen as an example of the number of herbal extracts that are tested in laboratories for antiviral properties. The search was obtained from MEDLINE using the terms 'herb' OR 'plant' AND 'antiviral' AND 'herpes simplex virus' and contained 137 references. Abstracts only were read and those containing information on testing of individual extracts, not mixtures, were included in this part of the study. Seventy abstracts were identified using these criteria, six contained information on animal testing (Table 1) and one reported a clinical trial [2]. Twenty-seven abstracts reported examination of activity against other viruses as well as herpes simplex types 1 or 2, or both. Forty-five authors described testing extracts of only one plant, 25 examined more than one plant and three of these examined more than 100 extracts.

Clinical trials

Although the scientific literature contains reports of laboratory testing of many hundreds of herbs or plants for their antiviral activity, our searches identified only 11 plant species (41 studies) that met the criteria of this review and had been used in clinical trials.

Phyllanthus spp.

Extracts of plants of the genus *Phyllanthus* have been used in traditional and folk medicine to treat jaundice and liver disease, and as a liver tonic [3,4]. This led to its selection at the Fox Chase Cancer Center, Pa., USA, for testing as a possible source of compounds with activity against hepatitis B virus (HBV). Extracts of *Phyllanthus* have yielded compounds including alkaloids, flavonoids, lignans, phenols, tannins and terpenes [5]. The mechanism of action against hepatitis B, involving disruption of hepatitis B polymerase, mRNA transcription and replication, has been elucidated using tissue culture, transgenic mice and woodchucks [6,7].

Our searches revealed 14 clinical trials, 13 of which were randomized, using *Phyllanthus* extracts for treatment of acute or chronic HBV infections (Tables 2 and 3). Seven of these trials reported some benefit from the use of this therapy, though six of these were of low methodological quality (Jadad score 1–2).

The first and most emphatically positive results were reported by Thyagarajan *et al.* from a group of 78 patients with chronic hepatitis B treated for 30 days with 0.6 g/day of *P. amarus* extract or placebo [8]. Clearance of hepatitis B surface antigen (HBsAg) was achieved in 59% of treated patients and in only 4% of controls. Subsequently, the same group reported an open trial with 28 symptomless carriers of HBV who were treated over 3 months with 0.75 g/day of *P. amarus* [9]. Sera were screened for HBV serological markers every month during treatment and for up to a year thereafter. Only four out of 20 individuals completing this trial cleared HBsAg.

A randomized clinical trial (RCT) in Thailand also failed to confirm the efficacy of *P. amarus* for treatment of chronic HBV infection [10]. One hundred and sixteen subjects received either 1.2 g/day of the herbal medicine or a placebo for 30 days. Examination of serological markers continued until 6 months from commencement of the trial and showed no significant changes.

Another 65 asymptomatic carriers of HBsAg in Thailand took part in a 30-day RCT and 42 subjects (20 from verum and 22 from placebo group) continued in a follow-up open study for a further 30 days [11]. The dose of *P. amarus* used in the RCT was 0.6 g/day and this was doubled in the second part of the study. No significant changes in serological markers of HBV were observed.

A double-blind cross-over study designed by Berk *et al.* used a dose of 0.6 g/day of *P. amarus* for 28 days either preceded or followed by 28 days administration of an indistinguishable placebo preparation [12]. Participants had all been HBsAg-positive for more than 1 year and measurement of HBV serological markers continued for 12 weeks from start of therapy but no significant changes were observed.

Milne *et al.* assayed and standardized levels of the antiviral agent geraniin in *P. amarus* plant extracts before using doses of 0.87 g/day (60 mg geraniin) for 8 weeks in a RCT in New Zealand [13]. The authors also measured levels of geraniin in the preparation successfully used by Thayagaran *et al.* [8] and found it to contain 36 mg/day. One hundred and five HBsAg carriers were enrolled and neither verum nor placebo group showed significant changes in serum levels of HBV serological markers or other biochemical markers of liver disease.

In China a group of 123 patients with chronic HBV infection took part in a RCT comparing three different

Table 1. Antiviral plant extracts: animal testing against herpes simplex virus

Author (year)	Virus	Plants	Animal	Route of infection	Comment	Ref.
Nakano (1998)	HSV-2	<i>Rhus javanica</i>	Guinea pigs	Intravaginal	Reduced incidence, severity and/or recurrence of lesions	[66]
Serkedjjeva (1999)	HSV-1	<i>Geranium sanguineum</i> L.	Guinea pigs	Cutaneous	Delayed development of vesicles	[67]
Kurokawa (1999)	HSV-1	<i>Rhus javanica</i> (purified acids)	Mice	Cutaneous	Moronic acid showed therapeutic efficacy	[68]
Nawawi (1999)	HSV-1	<i>Melaleuca leucadendron</i> , <i>Nephelium lappaceum</i>	Mice	Cutaneous	Delayed development of lesions and reduced mortality	[69]
Alche (2000)	HSV-1	<i>Melia azedarach</i> L.	Mice	Corneal	Reduced disease and corneal damage	[70]
Nawawi (2001)	HSV-1	<i>Stephania cepharantha</i>	Mice	Cutaneous	Reduced lesions and prolonged mean survival time	[71]

extracts, two from China and one from India [14]. Treatment continued for 3 months with doses of 0.9–2.7 g/day. The Indian extract of *P. amarus* appeared to be less effective but, because of limited supplies of the plant material, the group consisted of only 11 patients while there were 35 and 42 patients in the other treated groups. Patients treated with Chinese *P. urinaria* extracts were reported to have significantly increased clearance of HBeAg and production of HBe antibody.

Six other RCTs conducted in China reported use of extracts of *Phyllanthus* spp. for treatment of chronic HBV infections and only one reported no significant effect. Peng *et al.* (30 participants) reported no significant differences between patients treated with 1.2 g/day *P. amarus* or with placebo [15]. Two trials compared *Phyllanthus* extracts with other herbs and both Cao *et al.* (52 participants) [16] and Zhang *et al.* (130 participants) [17] recorded significant reductions in HBV serological markers in the *Phyllanthus*-treated groups. Other control groups received non-specific treatments in trials conducted by Huang (70 participants) [18] and Huang *et al.* (122 participants) [19], and polyinosinic and cytidylic acid in a trial conducted by Zhu *et al.* (75 participants) [20].

One double-blind RCT with 57 participants reported the use of *P. amarus* at a dose of 2.7 g/day over 5 days for treatment of acute hepatitis B [21]. The duration of disease as measured by the time taken for bilirubin level to come to below 2 mg% was used as the principal outcome measure. No significant difference was found between treatment and placebo groups.

Melissa officinalis L.

The active antiviral constituents of *Melissa officinalis* L appear to be the polyphenols and tannins [22], and activity against smallpox, mumps, Newcastle disease and herpes viruses has been demonstrated *in vitro*.

Natural recovery from herpetic infection of the skin usually occurs within 10–14 days but the early stages are very painful and some individuals have frequent

recurrences. Two double-blind RCTs have reported positive results from the use of a cream containing 1% dried melissa leaves extract for treatment of herpes simplex lesions. Wobling and Leonhardt conducted a pilot study (115 patients) followed by a double-blind RCT (116 patients) and demonstrated faster healing of herpes simplex lesions of the skin or transitional mucosa treated with melissa cream applied two to five times daily [23].

Sixty-six patients with recurrent herpes labialis were recruited by Koytchev *et al.* and instructed to commence treatment within 4 h of experiencing symptoms and to apply cream four times daily [2]. A significant reduction in symptom scores was recorded in the verum group, particularly on day 2 of treatment. Both patients and physicians were involved in scoring symptoms in these trials. Instituting treatment in the very early stages of infection improved speed of healing.

Sambucus nigra L.

Extracts of various parts of *Sambucus* spp. have their place in folk medicine and contain potential antivirals such as flavonoids, triterpenes, tannins and phenolic acids [22].

In vitro testing of a standardized preparation based on the berries of the black elder indicated anti-influenza virus properties and led to its use in a double-blind, placebo-controlled RCT during an influenza B epidemic [24]. The study group consisted of 40 individuals and most showed serological evidence of influenza B infection. Children received two and adults four tablespoons per day of either standardized elderberry syrup (Sambucol®) or placebo. In the treated group, higher mean antibody titres in convalescent-phase sera indicated an enhanced immune response to the virus, though this did not reach the level of significance. There was, however, significantly faster recovery in the treated group.

Similar positive results have recently been obtained from a further RCT, details of which have been extracted from an abstract [25]. Sixty patients suffering from flu-like symptoms for less than 48 h

Table 2. Antiviral plant extracts: randomized controlled clinical trials

Author (year)	Jadad score	Sample size, duration of trial	Herbal medicine tested	Condition treated	Experimental treatment	Control/comparative treatment	Primary outcome measures	Main results	Ref.
Thyagarajan (1988)	3	78, 30 days	<i>Phyllanthus amarus</i>	Chronic hepatitis B	Whole plant dried, root removed, ground, encapsulated 0.6 g/day	Placebo	Changes in levels of HBsAg, HBeAg, anti-HBc IgM	59% treated cleared HBsAg vs 4% controls	[8]
Leelaramee (1990)	2	116, 30 days	<i>Phyllanthus amarus</i>	Chronic hepatitis B	Whole plant dried, root removed, ground, encapsulated 1.2 g/day	Placebo	Changes in levels of HBsAg, HBeAg	No significant intergroup differences	[10]
Thamlikittikul (1991)	3	65, 30 days	<i>Phyllanthus amarus</i>	Chronic hepatitis B	Dried, root removed, ground, encapsulated 0.6 g/day	Placebo	Changes in levels of HBsAg	No significant intergroup differences	[11]
Berk (1991)	4	10, 28 days (cross-over)	<i>Phyllanthus amarus</i>	Chronic hepatitis B	Freeze-dried, ground, encapsulated 0.2 g/day	Placebo	Changes in levels of HBsAg, HBeAg, HBV DNA	No changes in outcome measures	[12]
Zhang (1992)	1	130, 90 days	<i>Phyllanthus amarus</i>	Chronic hepatitis B	Whole plant, decocted as oral liquid 60 g/day	Radix <i>Isatidis seu baphicacanthi</i>	Changes in levels of HBsAg, HBeAg	Significant reduction in HBeAg in treated group	[17]
Zhu (1992)	1	75, 90 days	<i>Phyllanthus urinaria</i>	Chronic hepatitis B	Whole plant, decocted as oral liquid 50 g/day	Polyinosinic and cytidylic acid	Changes in levels of HBsAg, HBeAg	Significant reduction in HBeAg in treated group	[20]
Peng (1993)	2	30, 60 days	<i>Phyllanthus amarus</i>	Chronic hepatitis B	Dried, root removed, ground, encapsulated 1.2 g/day	Placebo	Changes in levels of HBsAg, HBeAg	No significant intergroup differences	[15]
Huang (1993)	1	122, 30 days	<i>Phyllanthus amarus</i>	Chronic hepatitis B	Whole plant, decocted, filtered, concentrated as syrup 15 g plant material equivalent/day	Vitamins, glucoside	Changes in levels of HBsAg, HBeAg	Significant reduction in HBeAg in treated group	[19]
Milne (1994)	3	105, 8 weeks	<i>Phyllanthus amarus</i>	Chronic hepatitis B	Extracted with acetone/water, concentrated, dried, encapsulated 0.87 g/day	Placebo	Changes in levels of HBsAg, HBeAg	No significant intergroup differences	[13]
Wang (1995)	1	123, 3 months	<i>Phyllanthus amarus</i> (India) <i>P. niruri</i> (China) <i>P. urinaria</i> (China)	Chronic hepatitis B	Dried, ground, encapsulated 1.5 g/day (<i>P. amarus</i>) 0.9 g/day month 1, 1.8 g/day month 2 2.7 g/day month 3 (<i>P. niruri</i> , <i>P. urinaria</i>)	No herbal treatment	Changes in levels of HBsAg, HBeAb, HBeAg, HBeAb	Significant increase in patients eliminating HBeAg and developing HBeAb in <i>P. urinaria</i> group	[14]

Table 2. Antiviral plant extracts: randomized controlled clinical trials (cont.)

Author (year)	Jaded score	Sample size, duration of trial	Herbal medicine tested	Condition treated	Experimental treatment	Control/comparative treatment	Primary outcome measures	Main results	Ref.
Cao (1998)	1	52, 90 days	<i>Phyllanthus urinaria</i>	Chronic hepatitis B	Whole plant, dried, ground, encapsulated 1.2 g/day	Rhizoma <i>Polygoni cuspidati</i> , Ramulus <i>taxilli</i> , Radix <i>Astragal</i>	Changes in levels of HBsAg, HBeAg, HBV DNA, HBV DNAP	Significant reduction in HBsAg, HBV DNA, HBV DNAP in treated group	[16]
Huang (1999)	1	70, 90 days	<i>Phyllanthus urinaria</i>	Chronic hepatitis B	Whole plant, decocted as oral liquid 200 ml (40 g)/day	Intravenous vitamin C and glucoside in 10% dextrose	Changes in levels of HBsAg, HBeAg, HBV DNA, liver function tests	Significant reduction in HBV DNA in treated group, improvement in liver function	[18]
Narendranathan (1999)	5	57, 1 week	<i>Phyllanthus amarus</i>	Acute hepatitis B	Whole plant, dried, ground, encapsulated 2.7 g/day	Placebo	Time to reduce bilirubin to 2 mg%	No significant intergroup difference	[21]
Wöbling (1994)	4	116, 5–10 days	<i>Melissa officinalis</i> L	Herpes simplex infection <72 h duration	1% dried extract from leaves in cream base applied 2–4 times a day	Placebo	Symptoms score on day 2 and on termination of treatment	Significant reduction in symptom score in treated group on day 2	[23]
Koytchev (1999)	4	66, 5 days	<i>Melissa officinalis</i> L	Herpes labialis ≥ 4 episodes/year, trial episode <4 h duration	1% dried extract from leaves in cream base 2 mm applied 4 times a day	Placebo	Daily score of symptoms	Significant reduction of mean daily score in treated group on day 2	[2]
Zakay-Rones (1995)	5	40, 3 days	<i>Sambucus nigra</i> L	Influenza B	Standardized syrup, 2–4 tbsp/day	Placebo	Daily recording of fever and symptoms	Significantly faster recovery in treated group	[24]
Thom (2002)	$\geq 3^*$	60, 5 days	<i>Sambucus nigra</i> L	Influenza A	Elderberry syrup, 15 ml 4 times a day	Placebo	Symptoms scored by patients 4 times a day during treatment, 2 times a day for 9 days after treatment	Influenza symptoms disappeared 4 days earlier in treated group	[25]
Melchior (1997)	3	50, 5 days	<i>Andrographis paniculata</i>	Common cold	Standardized tablets, 1.02 g/day	Placebo	Visual analogue scale assessment of symptoms, days sick leave	Significant reduction in symptoms and in days sick leave in treated group	[32]
Caceres (1999)	5	208, 5 days	<i>Andrographis paniculata</i>	Common cold	Standardized tablets, 1.2 g/day	Placebo	Visual analogue scale assessment of symptoms	Significant reduction in intensity of symptoms in treated group	[31]
Sangkitiporn (1995)	2	60, 7–14 days	<i>Clinacanthus nutans</i>	Herpes zoster	5% cream applied 5 times daily	Placebo	Pain score, time to crusting and complete healing	Lesion healing significantly faster in treated group	[35]

Table 2. Antiviral plant extracts: randomized controlled clinical trials (cont.)

Author (year)	Jadad score	Sample size, duration of trial	Herbal medicine tested	Condition treated	Experimental treatment	Control/comparative treatment	Primary outcome measures	Main results	Ref.
Charuwichitra-tana (1996)	5	120, 1–26 days	<i>Climacanthus nutans</i>	Herpes zoster	5% cream applied 3 times daily	Placebo	Pain score, time to crusting and complete healing	Lesion healing significantly faster in treated group	[37]
Syed (1996)	4	120, max 2 weeks	<i>Aloe vera</i>	Genital herpes	a) 0.5% hydrophilic cream b) 0.5% gel 3 times daily for 5 consecutive days, max 30 applications in 2 weeks	Placebo	Mean days to healing, number of patients cured	Significantly shorter mean time to healing in group a), cured patient numbers greater in group a) than group b) or placebo	[39]
Syed (1997)	4	60, max 2 weeks	<i>Aloe vera</i>	Genital herpes	0.5% hydrophilic cream	Placebo	Mean days to healing, number of patients cured	Significantly shorter mean time to healing and cured patients at 2 weeks in treated group	[40]
Carson (2001)	3	20, 1–13 days	<i>Melaleuca alternifolia</i>	Recurrent herpes labialis	6% aqueous gel applied 5 times daily	Placebo	Time to lesion healing	No significant intergroup differences	[42]
Durant (1998)	5	145, 4–64 weeks	<i>Buxus sempervirens</i>	HIV	Standardized preparation in capsules a) 0.99 g/day b) 1.98 g/day	Placebo	Therapeutic failure: 1) AIDS 2) AIDS-related complex 3) CD4 count <2.0x10 ⁶ /l twice	Significant delay of progression to disease in group a)	[43]
Saller (2001)	5	149, 10–14 weeks	<i>Salvia officinalis</i>	Herpes labialis <24 h	Sage leaf extract cream 23 mg/g every 2–4 h during waking hours	a) acyclovir b) rhubarb-sage cream 23 mg/g	Time to complete healing, time to crust formation, symptom assessment	No significant intergroup differences	[49]
Josling (2001)	5	146, 12 weeks	<i>Allium sativum</i> L	Common cold (prevention and treatment)	Allicin-containing supplement 1 capsule/day	Placebo	Number of episodes of common cold, severity of symptoms	Significantly fewer colds of shorter duration in treated group	[46]
Suzuki (1983)	5	133, 4 weeks	<i>Glycyrrhiza glabra</i>	Chronic hepatitis	Intravenous, 40 ml SNMC/day	Placebo	Clinical improvement, liver function tests	Treated group showed significant overall improvement in clinical markers and in some liver function tests	[54]
Su (1984)	1	40, 30 days	<i>Glycyrrhiza glabra</i>	Acute hepatitis B	Capsules, 2x7.5 g crude glycyrrhizin equivalent per day, plus vitamins	Intramuscular inosine, and polyinosinic and cytaidylic acid, plus vitamins	Changes in HBsAg, HBeAg, liver function, IgA, Ig G, IgM	50% acute and chronic cases cleared HBsAg and HBeAg in treated group vs none of controls	[53]
		40, 90 days	<i>Glycyrrhiza glabra</i>	Chronic hepatitis B					

Table 2. Antiviral plant extracts: randomized controlled clinical trials (cont.)

Author (year)	Jadad score	Sample size, duration of trial	Herbal medicine tested	Condition treated	Experimental treatment	Control/comparative treatment	Primary outcome measures	Main results	Ref.
van Rossum (1999)	5	57, 4 weeks	<i>Glycyrrhiza glabra</i>	Chronic hepatitis C	Intravenous, 40, 80 or 120 ml SNMC 3 times a week	Placebo	Changes in ALT, HCV RNA	Mean decrease in ALT levels 26% in treated group, 6% in placebo	[59]
Tsubota (1999)	3	170, 24 weeks	<i>Glycyrrhiza glabra</i>	Chronic hepatitis C	100 ml SNMC 3 times a week plus UDCA 600 mg/day (Group A)	100 ml SNMC 3 times a week (Group B)	Changes in AST, ALT, GGT	Significantly greater reduction in all three parameters in Group B than in Group A	[56]
Iino (2001)	3	100, 6 weeks	<i>Glycyrrhiza glabra</i>	Chronic hepatitis B and C	100 ml SNMC/day for 3 weeks (Group A)	40 ml SNMC/day for 3 weeks (Group B)	Improvement in ALT levels	ALT levels in Group A significantly improved over levels in Group B ($P=0.005$)	[57]
Miyake (2002)	3	112, 12 weeks	<i>Glycyrrhiza glabra</i>	Chronic viral hepatitis	100 ml SNMC 3 times a week (Group A)	40 ml SNMC 3 times a week (Group B)	Improvement of ALT levels	Greater improvement ALT levels in Group A than Group B ($P=0.0002$)	[58]

*Insufficient detail in abstract.

were treated with 15 ml elderberry syrup or placebo four times per day for 5 days. Patients recorded and scored symptoms four times per day during treatment and twice per day for 9 days thereafter. In the elderberry-treated group symptoms disappeared an average of 4 days earlier than in the placebo group ($P < 0.001$). A therapeutic effect on flu-like symptoms was also indicated by significantly less use of rescue medication in the elderberry-treated group.

Andrographis paniculata

Andrographolide, a diterpene lactone isolated from *Andrographis paniculata*, has anti-inflammatory [26] and immunostimulant [27] properties, and dehydroandrographolide succinic acid monoester (DASM), from andrographolide, inhibits HIV *in vitro* [28]. In cell culture DASM interfered with HIV-induced cell fusion and with the binding of virus to the cells.

Clinical trials have reported the use of *A. paniculata* extracts for prevention [29] and treatment of common colds (Tables 2 and 3). In Chile a group of 61 participants with common cold symptoms were treated with 1.2 g per day of *A. paniculata* or placebo for 5 days in a double-blind study [30]. Total scores of clinical symptoms after 4 days treatment were significantly reduced in the verum group.

A further large double-blind RCT of *A. paniculata* extract for treatment of common colds was reported by the same group who recruited 208 individuals [31]. For social reasons 50 (23 verum, 27 placebo) of the group did not continue in the study but the remaining 158 were treated with 1.2 g of dried *A. paniculata* extract or placebo daily for 5 days and completed self assessment symptom evaluation forms. Data presented included statistical analyses of both intention-to-treat and treated groups and the authors stated that even 'worst case' analysis showed a significant difference in favour of the treatment.

Fifty subjects with cold symptoms were recruited into a double-blind RCT by Melchoir *et al.* and given standardized Kanjang® (85 mg extract) or placebo tablets with instructions to take four tablets three times daily for 5 days [32]. Patients kept a temperature and symptom record for 5 days and then returned to the clinic for assessment. Significant reduction in symptoms and in days sick leave were reported in the treated group.

An escalating dose trial designed primarily to assess safety and tolerability of andrographolide from *A. paniculata* and also possible anti-HIV effects, was terminated early because of adverse events [33]. Adverse events were defined as all disorders of well being and most participants reported at least one. Most were considered to be mild or moderate but one HIV-positive subject experienced an anaphylactic reaction

in week 4 of the study. Five of the 18 participants were healthy HIV-negative volunteers but, in the HIV-positive group, a significant rise in mean CD4 lymphocytes was observed after administration of 10 mg/kg andrographolide. There were no statistically significant changes in mean plasma HIV-1 RNA levels.

Clinacanthus nutans

Sulphur-containing glucosides, C-glycosyl flavones and lupeol have been isolated from *Clinacanthus nutans*, which is used in Thai traditional medicine [34]. Extracts have exhibited virucidal effects against some members of the herpes virus family *in vitro* [35], and one non-randomized and two RCTs in Thailand have reported positive results from the use of a 5% *C. nutans* cream on genital herpes and on herpes zoster lesions (Tables 2 and 3).

A clinical trial in which 77 patients with early genital herpes lesions applied 5% *C. nutans*, 5% acyclovir (Zovirax®) or placebo creams four times daily for up to 10 days was reported by Jayavasu *et al.* [36]. Participants revisited their doctors on days 4, 7 and 14 of the trial and supplied samples for virological and haematological testing. Use of either *C. nutans* or acyclovir creams was associated with significantly faster healing of lesions than the placebo cream.

Sankitporn *et al.* conducted a RCT with 60 participants, suffering from herpes zoster infections, applying *C. nutans* or placebo creams five times daily for 7–14 days. More rapid crusting and healing of lesions was observed in the treated group [35]. Similar results were recorded by Charuwichitratana *et al.* in a double-blind RCT with 120 herpes zoster patients who applied cream three times per day until lesions were completely healed [37].

Aloe vera

Aloe vera contains many potentially active constituents including vitamins, enzymes, minerals, sugars, lignin, saponins, salicylic and amino acids. Preparations have been tested in clinical trials for skin conditions as well as diabetes and hyperlipidaemia [38]. Syed *et al.* have conducted two trials of *A. vera* in treatment of primary genital herpes infections in men [39,40]. The first trial included 120 men randomized into three parallel groups to test clinical efficacy and tolerability of 0.5% *A. vera* extract in hydrophilic cream or gel carriers, against a placebo preparation [39]. Test products were applied three times daily for 5 consecutive days per week, with a maximum of 30 applications in 2 weeks. Both *A. vera* preparations were effective in healing and reducing duration of herpetic lesions but healing time was significantly shortened in patients using the hydrophilic cream preparation (4.8 vs 7.0 and 14.0 days for gel and placebo, respectively). Cure rates of

Table 3. Antiviral plant extracts: other non-randomized clinical trials

Author (year)	Jadad score	Sample size, duration of trial	Herbal medicine tested	Condition treated	Experimental treatment	Control/comparative treatment	Outcome measures	Main results	Ref.
Thyagarajan (1990)	1	28, 3 months	<i>Phyllanthus amarus</i>	Chronic hepatitis B	Encapsulated 0.75 g/day	None	Changes in levels of HBsAg, anti-HBc IgM	20% cleared HBsAg	[9]
Hancke (1995)	3	61, 5 days	<i>Andrographis paniculata</i>	Common cold	1.2 g/day	Placebo	Symptom scores using visual analogue scale	Reduced symptoms and faster recovery in treated group	[30]
Calabrese (2000)	1	18, 9 weeks	<i>Andrographis paniculata</i>	HIV	Escalating dose 5–20 mg/kg	None	Changes in levels of CD4 HIV-1 RNA	Significant rise in CD4 levels after 10 mg/kg, trial interrupted at 6 weeks due to adverse events	[33]
Jayvasu (1992)	1	77, 6–10 days	<i>Clinacanthus nutans</i>	Genital herpes	5% cream applied daily	a) acyclovir b) placebo	Time to crusting and complete healing	Lesion healing significantly faster in groups treated with <i>C. nutans</i> or acyclovir than in placebo group	[36]
Wöbling (1994)	1	115, 5–14 days	<i>Melissa officinalis</i> L	Herpes simplex infection <72 h duration	1% dried extract from leaves in cream base applied 5 times a day	None	Symptoms score on days 4, 6, 8 and on termination of treatment	Healing complete by day 8 in 96% (natural recovery usually 10–14 days)	[23]
Gotoh (1987)	1	11, 8 weeks	<i>Glycyrrhiza glabra</i>	HIV	5 ml/kg SNMC to maximum 400 ml daily for 3 weeks, then 3 times a week for 4–8 weeks	None	Changes in immune function, objective clinical improvement	Some improvement in asymptomatic carriers, none in AIDS patients	[61]
Mori (1989)	1	36, 11 weeks	<i>Glycyrrhiza glabra</i>	HIV	100–200 ml SNMC daily for 3 weeks then every second day for 8 weeks	400–800 ml daily for 3 weeks then every second day for 8 weeks	Changes in mitogenic responsiveness, liver function	Improved mitogenic responsiveness and liver function in both groups	[62]
van Rossum (2001)	1	15, 4 weeks	<i>Glycyrrhiza glabra</i>	Hepatitis C	100 ml SNMC 6 times/week	None	Changes in ALT levels	Mean change in ALT levels 47%	[60]

75, 45 and 7.5% were obtained in the cream, gel and placebo groups, respectively. There were no complaints of any drug-related side effects or hypersensitivity.

The second trial involved 60 men and compared two parallel groups treated with 0.5% *A. vera* extract in hydrophilic cream base or with the cream base only [40]. The protocol followed was the same as in the previous trial and 33.3% of patients using the active cream were cured after 1 week. Overall, the treated group had a significantly shorter mean time to healing (4.9 vs 12 days; $P < 0.01$).

Melaleuca alternifolia

The essential oil of *Melaleuca alternifolia*, commonly known as tea tree oil (TTO), is widely used as a topical antiseptic. Its chemical constituents include terpenes, it has broad antimicrobial properties and laboratory tests in tissue culture have demonstrated a direct antiviral effect [41]. A pilot clinical trial (20 patients), applying 6% TTO in aqueous gel or placebo gel five times daily for treatment of herpes labialis, has indicated some benefit, though not sufficient to reach statistical significance [42].

Buxus sempervirens L

Buxus sempervirens (boxwood) contains alkaloids and flavonoids but, prior to clinical trials, we found no published reports of its antiviral activity. An interesting case report led Durant *et al.* to conduct a pilot trial and subsequent RCT in 145 HIV- infected patients [43]. Two different doses (0.99 or 1.98 g/day) of a preparation of *B. sempervirens* were compared with placebo over 38 weeks. A small but significant increase in the time taken for CD4 cell counts to reach 'therapeutic failure' level of $< 2.0 \times 10^6/l$ was observed in the group treated with the lower dose of *B. sempervirens*.

Allium sativum L

The biological activities of *Allium sativum* (garlic) are thought to be due to its organosulphur compounds including allicin [22]. Extracts have shown *in vitro* activity against a number of human viruses [44,45] and one recent RCT has reported the use of a garlic supplement containing only stabilized allicin, for the prevention and treatment of the common cold [46]. One hundred and forty six volunteers participated for 12 weeks and the group treated with one allicin-containing capsule per day had significantly fewer colds (24 vs 65, $P < 0.001$) of shorter duration (1.52 vs 5.01 days, $P < 0.001$) than those in the placebo group.

Salvia officinalis L

Extracts of *Salvia officinalis* (sage) contain phenolic acids, tannins and volatile oils, and have antimicrobial effects [47]. The major constituents of *Rheum*

palmatum L (rhubarb) are anthraquinones and tannins. Saller *et al.* reported a screening study of plant extracts, for those exhibiting activity against herpes simplex virus, indicated that both rhubarb root and sage leaf extracts possessed such activity [48]. A pilot RCT (66 patients), comparing rhubarb root extract with conventional anti-herpes cream, acyclovir, indicated similar efficacy in treatment of herpes labialis (report cited in [49]). A further comparative RCT (149 patients) was then undertaken in eight centres, testing creams containing a mixture of rhubarb root and sage extracts (23 mg/g), sage extract (23 mg/g) alone and acyclovir cream (50 mg/g). Participants applied the product to the affected area every 2–4 h while awake for 10–14 days or until lesions were judged to be healed by patient or doctor. Again, there were no statistically significant differences between the groups though there was a trend of superiority of acyclovir over rhubarb-sage cream and of rhubarb-sage over sage-only cream.

Glycyrrhiza spp.

Glycyrrhizin is a terpenoid obtained by aqueous extraction from licorice root, *Glycyrrhiza glabra*, and in Japan has been an accepted treatment for chronic hepatitis for many years [50]. *In vitro* inhibition of growth of several DNA and RNA viruses, and irreversible inactivation of herpes simplex virus was reported in 1979 [51] and the mechanism of action on hepatitis A virus was investigated by Crance *et al.* [52].

Glycyrrhizin also appears to work as a free radical scavenger and has immunomodulatory effects. It is usually administered intravenously as Stronger Neo Minophagen C (SNMC), a solution containing 2 mg glycyrrhizin, 1 mg cysteine and 20 mg glycine per ml. Glycine is added to prevent pseudo-aldosteronism and cysteine to assist with liver detoxification.

Six RCTs and a follow-up open trial, reporting the use of glycyrrhizin for treatment of hepatitis, were located. Su *et al.* [53] used an in-house glycyrrhizin preparation administered orally, while all other trials used SNMC administered in single injections or infusions.

Suzuki *et al.* conducted a multicentre RCT involving 133 patients suffering from chronic hepatitis [54]. Those treated daily with 40 ml SNMC intravenously for 4 weeks showed significantly greater improvement clinically ($P < 0.001$) than the control group. Markers of liver function improved in both groups by the end of treatment though much more rapidly in treated than in the control group. The authors suggested that hospitalization may have been responsible for the improvement in the control group.

Twenty cases of acute hepatitis B and 20 cases of chronic hepatitis B were included in a RCT conducted by Su *et al.* [53]. The experimental group received an

in-house preparation of glycyrrhizin in capsules described as each containing the equivalent of 7.5 g of crude drug. The control group received intramuscular inosine (100 mg) daily with polyinosinic and cytidylic acids (2 mg) twice weekly. Acute cases were treated for 30 days and chronic cases for 90 days and both groups also received vitamins C, K and E. Liver function tests (LFTs) were performed pre- and post-trial in patients with acute hepatitis and every month in patients with chronic disease. There was no follow-up period but the degree of improvement in LFTs at the end of the trial was reported to be greater in the glycyrrhizin-treated group. Of patients originally HBsAg- and HBeAg-positive, 50% in the glycyrrhizin-treated group and none in the control group cleared the antigens.

In 1997, Arase *et al.* published a retrospective analysis of hepatitis C patients given long term treatment with SNMC compared to other herbal treatments and concluded that it was effective in preventing liver cancer [55]. This has encouraged further clinical trials to attempt to confirm the most effective dosage and treatment schedule. In Japan three comparative RCTs have examined 40 and 100 ml SNMC administered daily or three times per week over periods from 3 to 24 weeks in patients with chronic hepatitis predominantly caused by hepatitis C virus (HCV).

Tsubota *et al.* compared a regimen of 100 ml SNMC three times per week alone or supplemented with 600 mg per day ursodeoxycholic acid (UDCA) in 164 patients attending a single hospital [56]. Clinical and laboratory assessments were carried out every 4 weeks for 8 weeks prior to the trial, during the 24 week treatment phase and for 8 weeks after treatment. Normalization of levels of serum aspartate transaminase (AST) and γ -glutamyl transpeptidase (γ -GTP) occurred significantly more frequently in patients supplemented with UDCA. Similar numbers in both groups had normalization of alanine transaminase (ALT) levels.

In a multicentre RCT 178 patients with biopsy evidence of chronic hepatitis or liver cirrhosis were treated with 40 ml SNMC per day initially for 2 weeks [57]. One hundred of those whose ALT levels remained at levels ≥ 1.5 -times the upper limit of normal after 2 weeks were randomized to continue to receive that dose or to receive 100 ml SNMC daily for 3 further weeks. ALT levels were measured weekly and mean improvement in these levels assessed 1 week after treatment ended. The degree of improvement of ALT levels was significantly greater in the group receiving the higher dose of SNMC ($P=0.005$).

Doses of 40 or 100 ml SNMC administered three times per week for 12 weeks have also been compared in a group of 112 patients in another multicentre RCT [58]. LFTs were performed every 2 weeks in the 4 weeks prior to treatment and then every 4 weeks until the end

of the trial. Mean reductions in ALT and AST levels at the end of trial compared to baseline were significantly greater for patients treated with 100 ml SNMC ($P=0.0002$ for ALT, $P=0.0003$ for AST). There were no significant differences in changes in γ -GTP and other biochemical markers between the two groups.

Van Rossum *et al.* [59] conducted a placebo-controlled RCT in 57 Europeans with hepatitis C. The three treated groups receiving 40, 80 or 120 ml SNMC intravenously three times per week for 4 weeks. Mean serum ALT levels dropped 15% below baseline in the treated groups within two days of starting treatment ($P<0.02$). At the end of treatment the mean decrease was 26%, significantly higher than the placebo group (6%). No linear dose-response was observed and ALT returned to pretreatment levels in the 4-week follow-up period. Neither serum γ -GTP nor HCV RNA changed significantly in the course of the trial. A subsequent open trial was conducted to ascertain if administration of SNMC six times per week would be more effective in treatment of HCV [60]. This trial group consisted of 15 patients, 13 of whom had taken part in the in the RCT at least 6 months prior to enrolment in the open trial. All 15 patients received 100 ml SNMC six times per week for 4 weeks and there was a 4-week follow-up period. Mean percentage decrease in ALT levels at the end of treatment was 47% in this group, though these returned to pre-trial values by the end of follow-up. Also, minor reversible symptoms of pseudo-aldosteronism occurred in these patients.

Two small, uncontrolled trials reported the use of SNMC for treatment of HIV patients. Seven of the 11 patients treated by Gotoh *et al.* were asymptomatic carriers and this group showed some improvement, while patients with AIDS or AIDS-related complex did not [61]. Mori *et al.* administered SNMC to 36 HIV-positive haemophiliacs and considered it to be effective in preventing development of AIDS in asymptomatic carriers and in patients with AIDS-related complex [62].

Discussion

Many plant extracts have antiviral activity *in vitro*, but most are not suited for medicinal use in humans. The data summarized above show that 11 herbal extracts have been subjected to clinical trials. All plant species located by our searches (Tables 2 and 3) have shown some positive results in at least one clinical trial. Lack of standardization of extracts and dosage schedules make comparisons of individual trial results impossible. However, the conditions that might respond favourably to herbal antivirals include hepatitis, herpes simplex and zoster, influenza, common cold and HIV (Tables 2 and 3). These findings are encouraging and should stimulate further research.

Extracts of *Hypericum perforatum* L have also been reported to have antiviral effects against a range of human viruses *in vitro* and in animal experiments [63]. Two uncontrolled clinical trials testing the efficacy of hypericin in patients with HIV [64] and HCV [65] did not meet the criteria of this review because the hypericin preparations used were wholly synthetic. Neither trial demonstrated significant antiviral effects and a number of patients in both experienced phototoxic reactions.

A number of clinical trials of glycyrrhizin in the treatment of chronic hepatitis have produced encouraging results in some patients. Despite requiring hospital attendance for intravenous administration a minimum of 3 days per week, it has been generally well tolerated in trials lasting up to 24 weeks. However, all trials with follow-up have shown that improvements are not maintained when treatment is withdrawn. Development of an oral form that would be a much more convenient option for long-term therapy has been reported by van Rossum *et al.* [60].

It is unfortunate that much of the primary research is burdened with weaknesses. Liu *et al.* in a review of 22 RCTs using extracts of *Phyllanthus* spp. alone or in combination with other treatments concluded that the genus may have positive effects on clearance of HBV markers and on normalization of liver enzymes [4]. However, most trials were of low methodological quality and small sample size. No multicentre, large-scale RCT was identified and effects may be associated with less rigorous trials. The authors concluded that because of low quality and limited follow-up it was not possible to recommend *Phyllanthus* spp. for use in chronic hepatitis B.

Our systematic review has limitations as well. Even though our search strategy was comprehensive, we cannot be sure that all clinical trials have been located. Herbal medicine research is sometimes published in journals not readily accessible through electronic databases. Furthermore, negative trials may not be published at all. Thus, there is a danger that the overall impression created by this review is too positive.

Future studies in this area should be conducted to a high methodological standard. In particular, they should include a sufficiently large sample based on a power calculation, stringent entry criteria and validated outcome measures. In order to be reproducible it is mandatory to describe the specification of the herbal extract in full detail. Adverse events should be documented carefully. In order to answer the question of relative efficacy compared to a standard treatment, trials must be properly designed as equivalence studies.

In conclusion, the clinical trial data available to date show a considerable potential of herbal medicines as antiviral agents. This potential should be investigated in adequately designed studies.

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