

# A Review of the Experimental Approaches Used in Clinical Studies to Evaluate the Health Benefits of Plant Food Supplements Associated With Infectious Diseases

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## Abstract

The objective of this review was to evaluate the experimental approaches used in clinical trials to support the benefit claims of plant food supplements (PFS) with reported activity on infectious diseases. A literature search was conducted on a list of 309 plant species currently used in food supplements in Europe using the National Centre for Biotechnology Information (NCBI) PubMed Database to identify all the clinical trials for evaluating the benefit claim for preventing or treating infectious diseases in humans. The searches included a combination of terms related to the name of plants, class of infectious agents and therapeutic activities against infectious disease. By limiting the searches to clinical studies, only 27 articles representing 19 plant species were identified. From this list, 13 papers from the 10 plants most extensively researched were critically evaluated for assessing methods used to assess the benefits of PFS. Different study designs were used ranging from an open trial with no placebo or control and no randomization to double-blind randomized placebo controlled trials including a crossover design. Although the experimental approaches described in this review were found to be suitable for evaluating the benefit claims of PFS for treating infectious diseases caused by bacteria, viruses, fungi and parasites, the clinical study design should be more standardised as many studies lacked a control group and sufficient population size to be statistically acceptable taking into consideration patient variability. The reporting of the results varied and should also be standardised to include all the study parameters and data collected.

**Keywords:** anti-infectious, clinical studies, plant food supplements

## 1. Introduction

An infection is the colonization of a host organism such as a human by a parasitic organism/pathogen to reproduce, often resulting in the host's health being compromised. Infectious agents that affect humans include viruses, bacteria, prions, and fungi. Host organisms are normally able to fight infections via their immune systems. However, people with chronic infections or weak immune systems will often need external interventions such as treatments using anti-infective medicine (National Institutes of Health). Along with a wide range of synthetic and semi-synthetic antibiotics and antimicrobial agents, many food supplements (FS) and plant food supplements (PFS) have been registered on the European market with claims related to anti-infective activity. Plant based food supplements contain various classes of compounds that have been studied for their anti-infective activities (Cowan, 1999).

The Directive 2002/46/EC of the European Parliament and of the Council defines Food Supplements (FS) as the foodstuffs for which the purpose is to supplement the normal diet and that are concentrated sources of nutrients or other substances with a nutritional or physiological effect, alone or in combination, marketed in dose form, namely forms such as capsules, pastilles, tablets, pills and other similar forms, sachets of powder, ampoules of liquids, drop dispensing bottles, and other similar forms of liquids and powders designed to be taken in measured small unit quantities (EC, 2002). PFS is a FS in which the active ingredient/s is plant based. According to regulations PFS are registered as food products and therefore cannot have medical claims such as the prevention and treatment of a disease.

The objectives and endpoints of the clinical studies evaluating PFS should be carefully designed such that the data obtained can be used for making non-medicinal benefit claims. The specificity of the regulatory status of PFS requires specific studies to provide the evidence in support of claims, and efficacy and safety studies of their activity in human subjects are limited and often methodologically poor (Cassileth, Heitzer, & Wesa, 2009).

This review has been conducted to examine the appropriateness of the clinical study designs to evaluate the benefit claims of plant species to support the registration of PFS in Europe.

## **2. Research Design and Methods**

### *2.1 Search Strategy*

A non-systematic review was conducted of the published literature by searching the National Centre for Biotechnology Information (NCBI) PubMed Database for studies published from 1990 to 2010.

The search used a combination of terms related to the name of plants contained in the Euro FIRE Basis Database (Bioactive Substances in Food Information System) and the infectious agents and disease effects. Search terms included in the article title, abstract, keywords and MeSH terms were related to general infectious agents ('bacteria', 'fungal', 'virus', 'parasitic', 'microbial') and effects ('anti-bacterial', 'anti-fungal', 'antiviral', 'antiparasitic', 'antimicrobial'). Search terms were crossed sequentially with each individual plant name (Genus/Species/Popular term) contained within the Euro FIRE Basis database. All research study designs were included, and eligibility limited to the inclusion and exclusion criteria defined below.

### *2.2 Benefit and Biomarker Definition*

Consensus criteria used to define "benefit" in the context of food consumption was developed by consortium partners within Plant LIBRA (EC project number 245199); namely "the attainment of specific physiological objectives, such as reduction of risk factors for chronic diseases and the maintenance of the human homeostasis, which is the body's capability to physiologically regulate wellbeing and ensure stability and balance in response to changes in the external environment". The definition of biomarker was based on the Biomarkers Definitions Working Group, whereby a biomarker is "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention" (Biomarkers Definitions Working Group, 2001).

### *2.3 Inclusion Criteria*

Criteria for including the studies in this review had to:

- i) concern the clinical evaluation of PFS
- ii) include the evaluation of beneficial effects of PFS on biomarkers for treating infectious diseases
- iii) be published in the English Language.

To evaluate the eligibility of the studies to these inclusion criteria, the following definitions were used:

**Plant food supplements:** the definition of PFS was aligned with the EC Directive 2002/46 of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements (EC, 2002). This defines food supplements (FS) as "the foodstuffs for which the purpose is to supplement the normal diet and that are concentrated sources of nutrients or other substances with a nutritional or physiological effect, alone or in combination, marketed in dose form, namely forms such as capsules, pastilles, tablets, pills and other similar forms, sachets of powder, ampoules of liquids, drop dispensing bottles, and other similar forms of liquids and powders designed to be taken in measured small unit quantities. Plant Food Supplements (PFS) is a type of food supplement (FS) in which botanicals preparations are the main ingredients (EC, 2002);

**Clinical Evaluations:** clinical trials based on all study designs including case-control studies, nested case-control studies, cross-sectional studies, parallel- or cross-over trials; trials comprising subjects of all age, gender, and racial groups;

**Data provided for treating infections:** Disease susceptibility or predisposition, bacterial, fungal, viral or parasitic infection;

**Language:** publications documented in English.

### *2.4 Data Extraction and Quality Assessment*

Studies meeting the criteria for selection were extracted by three researchers into a spread-sheet that captured the following data:

bibliographic details: first author, journal title, publication year, publication volume and page details;

study characteristics: trial setting (location(s), study run-in, study duration specified as days or weeks, study follow-up period specified as days or weeks;

sample size: treatment group (number of patients per dose group), control group (number of patients per placebo group);

methodological quality: Jadad scoring (scored as zero or one per category if no information or relevant information respectively was provided) – i) the incorporation of randomised designs, where each study participant had the same chance of receiving each intervention and this could not be predicted by the investigators, ii) the use of blinding designs, where neither the investigator nor the study participant could identify the intervention being assessed, iii) detailed reference to participant withdrawals due to non-compliance or other reasons. In the event of no withdrawals, this needed to be stated in the article;

inclusion and exclusion criteria: based on participant age, diagnosis, health status and lifestyle;

characteristics of participants: age (by participant group), weight/BMI (by participant group), male to female ratio;

intervention: details of product name and manufacturer, plant name and part, processing and treatment of plant material, active drug substance, excipients used, country of origin, dosage form, dosage strength, drug substance manufacturer, positive control name, positive control concentration/dose, negative control name, plant material biomarkers, content in test material, analytical method used to confirm active;

exposure: treatment dose (per administration), administrations per day/week, total dose per day/week; control dose (placebo or active per administration), control administrations per day/week, total control dose per day/week;

primary outcome measures: treatment outcome, descriptive statistics of baseline for infectious diseases values, endpoint values, significance level, methodology / technology used;

secondary outcome measures: treatment outcome, descriptive statistics of baseline for infectious diseases values, endpoint values, significance level, methodology / technology used; adverse events – diagnosed or self-reported.

One researcher evaluated all the extracted data for this review.

### 3. Results

#### 3.1 Literature Search and Study Characteristics

The literature search methodology and results are summarised in Figure 1. The initial NCBI database search was conducted using the infectious diseases and therapeutic effect search terms crossed with the individual plant names and synonyms of the 309 plant species selected in this study. Only nineteen of the 309 plant species had articles describing their use in clinical studies. 2256 ‘Hits’ were obtained for the nineteen plant species, however, only twenty seven articles reported the clinical evaluation of those plant species.

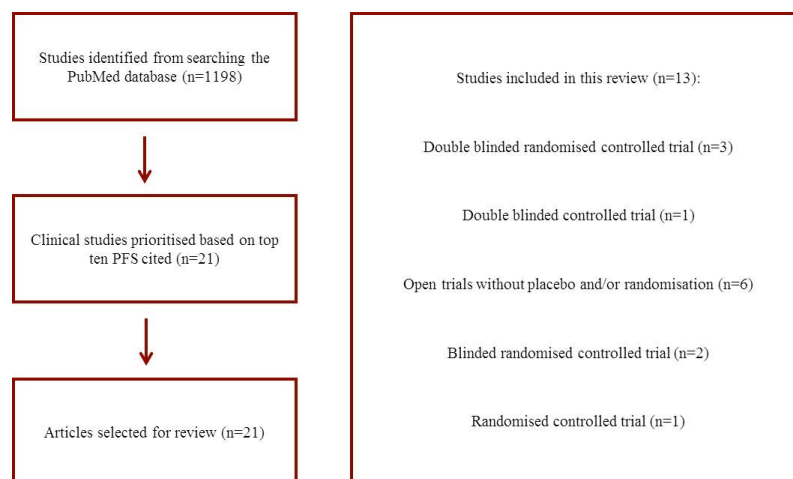


Figure 1. Selection process employed to identify trials and underlying methods designed to assess the benefits of plant food supplements for treating infectious diseases

Of the nineteen plants only ten plants had clinical studies published in English and for their effects on infectious diseases. These ten plants were selected for this review and are listed in Tables 1 and 2. 1198 'Hits' were obtained for the 10 plants, of which, twenty one articles reported the clinical evaluation of the PFS. One of the articles reported the clinical evaluation of two of the selected ten plants. The full article of each clinical study of the ten plants was retrieved for detailed examination of the clinical approaches used. Only 13 of the 21 articles were evaluated in this review since 2 articles were review articles, 1 article was published in the Chinese language, and 5 articles were not available from the source. Of the 13 articles clinically evaluating PFS, 6 reported anti-bacterial activity, 3 antifungal activity, 2 anti-parasitic activity and 2 anti-viral activity. Cranberry has been the most studied plant used in PFS for its anti-bacterial properties in treating urinary tract infections and shows activity against *Helicobacter pylori*, the bacteria that causes stomach ulcers.

Table 1. List of the ten plants used in food supplements with the most clinical studies published for their effects on infectious diseases

	<b>Plant used in food supplement</b>	<b>Reference</b>
1	<i>Vaccinium macrocarpon</i> Aiton ( <i>Oxycoccus macrocarpus</i> (Aiton) Pers., cranberry)	Bailey 2007, Shmuelly, 2007, Mcmurdo, 2005, Gotteland, 2008
2	<i>Allium sativum</i> (Garlic)	Davis 1990, Caporaso, 1983
3	<i>Piper longum</i> L. (Long pepper)	Agrawal, 1997
4	<i>Carica papaya</i> (Papaya / Paw Paw)	Okeniyi, 2007
5	<i>Cymbopogon citratus</i> (DC.) Stapf ( <i>Andropogon citratus</i> DC., Lemongrass)	Wright, 2009
6	<i>Citrus limon</i> ( <i>Citrus limonum</i> , Lemon)	Wright, 2009
7	<i>Trapa natans</i> L. (Water chestnut)	Hijikata, 2007
8	<i>Urtica dioica</i> L. (Stinging nettle)	Cai, 2009
9	<i>Ziziphus zizyphus</i> (L.) Meikle (jujube)	Maek-a-nantawat, 2009
10	<i>Prunus armeniaca</i> L. ( <i>Armeniaca vulgaris</i> Lam., Apricot)	Nakajima, 2006

Different study designs were used ranging from an open trial with no placebo or control and no randomization (Bailey, Dalton, Daugherty, & Tempesta, 2007; Hijikata, Yamada, & Yasuhara, 2007; Maek-a-nantawat, Phonrat, Dhitavat, Naksrisook, Muanaum, Ngamdee, & Pitisuttithum, 2009; Nakajima, Fujita, Inoue, Nishio, & Seto, 2006; Davis, Shen, & Cai, 1990; Caporaso, Smith, & Eng, 1983) to double blind randomized placebo controlled trial including crossover (Mcmurdo, Bissett, Price, Phillips, & Crombie, 2005; Gotteland et al., 2008).

The largest study contained 889 participants (Shmuelly, Yahav, Samra, Chodick, Koren, Niv, & Ofek, 2007) and smallest study contained 5 participants (Davis et al., 1990).

Two of the studies (Davis et al., 1990; Caporaso et al., 1983) conducted on garlic evaluating the anti-fungal potential were open trials with no placebo or control, no randomization, no inclusion and exclusion criteria and the population/sample size was too small (less than 10 participants).

Lumbar puncture and pathological profile of the stool were methods used to detect the fungi in the study by Davis et al. whereas analysis of serum and urine samples through culturing were means of detecting fungus content in the study by Caporaso et al. Due to the study design of these two trials being very poor (based on the methodological quality scoring of zero), the study results cannot be used to make the antifungal claims of garlic in PFS.

The randomized placebo controlled trial (Wright, Maree, & Sibanyoni, 2009) conducted on lemon and lemon grass PFS for evaluating their antifungal potential by treating oral thrush in HIV infected patients involved a population size of 90 participants. There were two treatment groups: one group was given lemon juice and the second group was given lemon grass infusion. The positive control group was given gentian violet aqueous solution 0.5% to apply topically inside the mouth. Oral thrush diagnosis as well as heart rate and blood pressure were monitored. This well-designed study scored 3 based on the methodological quality scoring (4 being the highest and 0 being the lowest score recorded).

Of the two studies evaluating the anti-parasitic potential of PFS, one was a double blind placebo controlled trial (Agrawal et al., 1997) while the other was a blind randomized placebo controlled trial (Okeniyi, Ogunlesi, Oyelami, & Adeyemi, 2007). The population size of both the studies ranged from 50 to 60 participants and used microscopic examination of the stool to measure the parasite content in patients. These study designs scored 2 based on the methodological quality scoring.

The two studies (Hijikata et al., 2007, Maek-a-nantawat et al., 2009) identified for evaluating the anti-viral potential of PFS investigated herbal mixtures and not individual plants. Both studies were open-label trials and scored low based on the methodological quality scoring. The study by Hijikata et al. included 28 participants and the Meares–Stamey test was used to measure *Herpes simplex* in two groups of patients (one group with herpes genitalis and one group with herpes labialis). The study by Maek-a-nantawat et al. involved a small population size of 18 participants with asymptomatic HIV, where the participants recorded all symptoms themselves on diary cards. CD4 cell counts, HIV viral loads and blood chemistry were monitored and questionnaires were used to assess quality of life. PFS should not be used to treat life threatening diseases such as HIV, however, this study was included in the review since the PFS was given in combination with anti-retrovirals to improve patient's health.

Of the six study trials identified for evaluating the anti-bacterial potential of PFS (Nakajima et al., 2006; Bailey et al., 2007; Cai et al., 2009; Shmueli et al., 2007; Mcmurdo et al., 2005; Gotteland et al., 2008), four were based on cranberry (Bailey et al., 2007; Shmueli et al., 2007; Mcmurdo et al., 2005; Gotteland et al., 2008). The study by Bailey et al. was an open label trial that measured urinary tract infections by urinalysis using a dipstick and microscopic examination. Only one group of 12 participants were treated by administering a capsule containing concentrated cranberry extract. The poor study design resulted in the study being given a score of zero.

Two studies (Shmueli et al., 2007; Mcmurdo et al., 2005) evaluating the anti-bacterial potential of cranberry juice were double blinded randomized placebo controlled trials. The one study (Shmueli et al., 2007) was a triple therapy to determine the additive effect of cranberry juice with a proton pump inhibitor and two antibiotics. One group was given cranberry juice with the antibiotic (89 participants), the second group was given the placebo juice with the antibiotic (88 participants), and the third group was only given the antibiotic (712 participants). The *Helicobacter pylori* bacteria were detected using the <sup>13</sup>C urea breath test and analysed by a mass spectrometer. The good study design and description scored this study a 4, which was the highest score given in this review. The study conducted by Mcmurdo et al. to evaluate the anti-bacterial potential of cranberry juice was a double-blind, placebo-controlled trial involving 376 participants. The urinary tract infection was measured by the urine dip stick test and the cultures were identified using the VITEK 1 (Biomérieux). The good study design and description scored this study a 3.

The multicentric double blind randomized placebo controlled trial (Gotteland et al., 2008) was designed to evaluate the additive or synergistic anti-bacterial potential of cranberry juice with the probiotic *Lactobacillus johnsonii* (La1) in children. The study involved 295 children and included three treatment groups and one control group that received juice over 3 weeks with 1 month washout period. The first treatment group one received cranberry juice with La1, the second treatment group received the placebo juice with La1; the third treatment group received cranberry juice with heat-killed La1; and the control group received placebo juice with heat-killed La1. The *Helicobacter pylori* bacteria were detected using the urea breath test and analysed by gas chromatography and the isotope ratio mass spectrometer. This study was given a methodological quality score of 3.

The study by Nakajima et al. evaluating the anti-bacterial potential of apricot was an open label trial that detected *Helicobacter pylori* using the <sup>13</sup>C urea breath test and measured with an infrared laser spectrometer. The study contained only one group of 18 participants that were given apricot juice as treatment. Due to the low patient sample number and poor study design the study was given a score of 1.

A blind randomized placebo controlled trial (Cai et al., 2009) was conducted using a population size of 143 participants to evaluate the anti-bacterial potential of PFS involving two treatment groups: one group given the prulifloxacin antibiotic and the other group given the prulifloxacin antibiotic together with other plant extracts including stinging nettle. The Meares–Stamey test was used to measure the chronic bacterial prostatitis. The study design and the large population size resulted in this study being given a score of 2.

### 3.2 Plant Food Supplements

The 10 plant species most commonly reported with clinical data to support the claimed use as anti-infectives in this study were:

*Vaccinium macrocarpon* Aiton (*Oxycoccus macrocarpus* (Aiton) Pers., cranberry) (Bailey et al., 2007, Shmueli et al., 2007, Mcmurdo et al., 2005, Gotteland et al., 2008), *Allium sativum* (Garlic) (Davis et al., 1990, Caporaso et al., 1983), *Piper longum* L. (Long pepper) (Agrawal et al., 1997), *Carica papaya* (Papaya / Paw Paw) (Okeniyi et al., 2007), *Cymbopogon citratus* (DC.) Stapf (*Andropogon citratus* DC., Lemongrass) (Wright et al., 2009), *Citrus limon* (*Citrus limonum*, Lemon) (Wright et al., 2009), *Trapanatans* L. (Water chestnut) (Hijikata et al., 2007), *Urtica dioica* L. (Stinging nettle) (Cai et al., 2009), *Ziziphus zizyphus* (L.) Meikle (jujube) (Maek-a-nantawat et al., 2009), *Prunus armeniaca* L. (*Armeniaca vulgaris* Lam., apricot) (Nakajima et al., 2006).

The PFS were administered either orally in different dosage forms (capsule or juice), or were administered intravenously. PFS formulations were either sourced from commercial manufacturers and suppliers (Hijikata et al., 2007; Maek-a-nantawat et al., 2009; Nakajima et al., 2006; Bailey et al., 2007; Shmueli et al., 2007; Mcmurdo et al., 2005; Gotteland et al., 2008), or the manufacturing source of the formulation was not stipulated (Antiparasitic: Agrawal et al., 1997, Okeniyi et al., 2007; Antifungal: Wright et al., 2009; Caporaso et al., 1983; Davis et al., 1990; Antibacterial: Cai et al., 2009).

### 3.3 Study Population, Adverse Events, Study Duration

The inclusion criteria for selecting the study population for the desired clinical outcomes varied across the different studies depending on the infectious agents (Table 2); these included participants that had the infection/disease for a specific time period, either had symptoms of the disease or tested positive in the preliminary screen. Many studies enrolled participants that were patients being treated at hospital. Two of the studies did not specify the inclusion criteria (Davis et al., 1990; Caporaso et al., 1983).

Table 2. Study characteristics

<b>Plant used in food supplement</b>	<i>Piper longum</i> L. (Long pepper)
<b>Study design (Quality score)</b>	Double blind placebo (2)
<b>Study Population and Inclusion Criteria</b>	25 treated, 25 placebo controls suffering from giardiasis with clinical signs and symptoms, and stools positive for trophozoites: cysts of <i>Giardia lamblia</i> .
<b>Intervention</b>	1g capsule containing Buteamonosperma and <i>Piper longum</i> was administered three times daily for a period of 15 days. Weekly follow up.
<b>Control</b>	Baseline measures
<b>Outcomes measures</b>	Pathological profile of stool observing changes in haematological parameters
<b>Significance</b>	After 15 days of drug treatment there was a complete disappearance of <i>G. Lamblia</i> (trophozoites:cysts) from the stools of 23 out of 25 patients. Symptoms of ill health and abdominal discomfort, presence of mucus, pus cells and rbc's were significantly reduced. There was a marked improvement in the clinical and haematological profile of the patients. Spontaneous recovery in 20% cases was recorded in placebo controls.
<b>Adverse events</b>	None reported.
<b>Plant used in food supplement</b>	<i>Vaccinium macrocarpon</i> Aiton ( <i>Oxycoccus macrocarpus</i> (Aiton) Pers., cranberry)
<b>Study design 1 (Quality score)</b>	Open label pilot study (0)
<b>Study Population and Inclusion Criteria</b>	12 participants. Criteria: History of a minimum of 6 UTIs in the preceding year.
<b>Intervention</b>	One capsule twice daily for 12 weeks containing 200 mg of a concentrated cranberry Extract standardized to 30% phenolics. Follow up after two years.
<b>Control</b>	No control

<b>Outcomes measures</b>	Urinalysis - dipstick and microscopy exam.  Dipstick test examined the specimen for red blood cells, nitrates, lymphocytes, protein, glucose, and measured specific gravity. The microscopic exam looked for red blood cells and white blood cells as well as bacteria and casts. Any samples with positive results were to be cultured and sensitivity toward antibiotics determined.
<b>Significance</b>	A cranberry preparation with a high phenolic content may completely prevent UTIs in women who are subject to recurrent infections.
<b>Adverse events</b>	None reported.
<b>Study design 2 (Quality score)</b>	Double-blind randomized trial (4)
<b>Study Population and Inclusion Criteria</b>	177 <i>H. Pylori</i> infected patients at the laboratory between 1998 and 2003 who were found to have a value of A3.5 on the 13C-UBT.
<b>Intervention</b>	One group of patients (712) were only given omeprazole, amoxicillin and clarithromycin (OAC) over the study period.  The second group of 89 patients were given OAC and 250 ml of cranberry juice twice daily for 1 week.  The third group of 88 patients were given OAC and 250 ml of placebo juice twice daily for 1 week.  The two groups that were given the juice were only given juice over the next two weeks without the OAC.
<b>Control</b>	Two control groups
<b>Outcomes measures</b>	<sup>13</sup> C urea breath test and mass spectrometry analysis.
<b>Significance</b>	The rate of <i>H. Pylori</i> eradication (13C-UBT a 3.5) was 82.5%, with no statistically significant difference among the three arms. Analysis by gender revealed that for female subjects, the eradication rate was higher in the cranberry-OAC arm (n = 42, 95.2%) than in the placebo-OAC arm (n = 53, 86.8%) and significantly higher than in the non-placebo-OAC group (n = 425, 80%; p = 0.03). For males, the rate was non significantly lower in the cranberry-OAC arm (n = 35, 73.9%) than in the placebo-OAC arm (n = 45, 80.0%) and non-placebo-OAC group (n =287, 85.0%). These results suggest that the addition of cranberry to triple therapy improves the rate of  <i>H. Pylori</i> eradication in females.
<b>Adverse events</b>	None reported.
<b>Study design 3 (Quality score)</b>	Randomised, placebo-controlled, double-blind trial (3)
<b>Study Population and Inclusion Criteria</b>	376patients admitted to either acute Medicine for the Elderly assessment or rehabilitation units in hospital in Tayside, Scotland
<b>Intervention</b>	187 participants were given 150 ml cranberry juice twice a day (32 of participants in the group were prescribed antibiotics for any indication during the period of observation),  189 participants were given 150 ml placebo juice twice a day (35 of participants in the group were prescribed antibiotics for any indication during the period of observation)
<b>Control</b>	Baseline measures
<b>Outcomes measures</b>	Secondary outcomes were adherence to beverage drinking, courses of antibiotics prescribed, and organisms responsible for UTIs.  Median [IQR] adherence from a maximum of 300 ml per day was good at 300 [44] ml for the placebo beverage and 300 [28]ml for the cranberry juice (P=0.208, Mann–Whitney Test).
<b>Significance</b>	The between-group differences were not significant, relative risk (RR) 0.51 [95% CI 0.21–1.22, P = 0.122). Although there were significantly fewer infections with <i>Escherichia coli</i> in the cranberry group (13 versus 4) RR 0.31 [95% CI 0.10–0.94, P =0.027], this was a secondary

	outcome.
<b>Adverse events</b>	13 adverse events occurred and all resulted in withdrawal from the study. The six in the placebo group comprised two deaths and four episodes of gastrointestinal upset. The seven in the cranberry group comprised three deaths, two episodes of gastrointestinal upset, one episode of skin redness and itching, and one elevated blood glucose level in a known diabetic patient.
<b>Study design 4 (Quality score)</b>	Multicentric, randomized, controlled, double-blind trial (3)
<b>Study Population and Inclusion Criteria</b>	295 asymptomatic children (6–16 y of age). Criteria: Positive 13C-urea breath test (UBT) result and without antecedents of gastrointestinal pathologies, chronic diseases, or a recent history of antibiotic, antacid, or prokinetic drug treatment.
<b>Intervention</b>	Four groups: 74 given cranberry juice with probiotic <i>Lactobacillus johnsonii</i> La1, 74 given placebo juice with probiotic <i>Lactobacillus johnsonii</i> La1, 73 given cranberry juice with heat-killed La1, and 74 given placebo juice with heat-killed La1 (control).  Cranberry Juice (200 ml) and La1 product (80 ml) were given daily for 3 weeks, after which a second UBT was carried out. A third UBT was done after a 1-month washout in those children who tested negative in the second UBT.
<b>Control</b>	Baseline measures
<b>Outcomes measures</b>	Result of the 13C-urea breath test after the 3 week treatment period.  Breath test with a DOB $\geq 5\%$ for the entire 30-min period was considered positive for H. Pylori.
<b>Significance</b>	Data analysis was done with Statistica 4.5 for Windows.  Helicobacter pylori eradication rates significantly differed in the four groups: 1.5% in the control group compared with 14.9%, 16.9%, and 22.9% in the La1, CB, and CB/La1 groups, respectively ( $P < 0.01$ ); the latter group showed a slight but not significant increase when compared with the other treated groups.  No synergistic inhibitory effects on H. Pylori colonization were observed when both foodstuffs were simultaneously consumed.
<b>Adverse events</b>	None reported.
<b>Plant used in food supplement</b>	<i>Allium sativum</i> (Garlic)
<b>Study design 1 (Quality score)</b>	Non randomised, no control or placebo, non blinded (0)
<b>Study Population and Inclusion Criteria</b>	No inclusion criteria reported. 5 participants: 2 – cryptococcal meningitis, 2 - viral meningitis, 1 - unknown chronic meningitis
<b>Intervention</b>	Commercial <i>Allium sativum</i> (garlic) extract was given intravenously (1 mg/kg of body weight dose).
<b>Control</b>	No control
<b>Outcomes measures</b>	Primary outcome measures: Lumbar puncture Secondary outcome measures: Pathological profile of stool. Biomarkers: diallylthiosulfinate (allicin), diallyldisulfide, and diallyltrisulfite
<b>Significance</b>	Plasma titers of anti-Cryptococcus neoformans activity rose two fold over pre-infusion titers. Anti-C. Neoformans activity was detected in four of five cerebrospinal fluid samples but not in pooled normal cerebrospinal fluid.
<b>Adverse events</b>	Adverse effects of i.v. Commercial A. Sativum administration occurred in less than 25% of patients and included abdominal discomfort, nausea, and thrombophlebitis at the I.v. Site. No cases of anaphylaxis were recognized. Abdominal discomfort and nausea appeared to depend



	on the rate of drug delivery.
<b>Study design 2 (Quality score)</b>	Non randomised, no control or placebo, non blinded (0)
<b>Study Population and Inclusion Criteria</b>	No inclusion criteria reported. 5 participants
<b>Intervention</b>	A fresh extract of garlic ( <i>Allium sativum</i> ) was administered orally
<b>Control</b>	No control
<b>Outcomes measures</b>	At intervals, serum and urine were collected and assayed for antifungal activity.
<b>Significance</b>	The maximum tolerable dose was determined to be 25 ml of garlic extract. After oral ingestion of 25 ml of the extract, anticandidal and anticryptococcal activities were detected in undiluted serum 0.5 and 1 h after ingestion. No detectable antifungal activity was found in the excreted urine at any time after oral ingestion. Oral garlic is of limited value in the therapy of human fungal infections.
<b>Adverse events</b>	In the volunteer who took a 25-ml dose of the extract, additional symptoms of nausea, diaphoresis, and light-headedness lasting For 30 min were noted. Larger amounts of garlic extract caused severe burning sensations in the esophagus and the stomach and vomiting.
<b>Plant used in food supplement</b>	<i>Carica papaya</i> (Papaya / Paw Paw)
<b>Study design (Quality score)</b>	Randomized blind placebo (2)
<b>Study Population and Inclusion Criteria</b>	60 children Criteria: Normal weight and height for age, no health complaints, stool microscopic evidence of intestinal parasites
<b>Intervention</b>	20 ml elixir (seeds in honey) given once a day.
<b>Control</b>	Baseline measures
<b>Outcomes measures</b>	Microscopic examination of stool
<b>Significance</b>	The stool clearance rate for the various types of parasites encountered was between 71.4% and 100% following CPH elixir treatment compared with 0–15.4% with honey. Air-dried <i>C. Papaya</i> seeds are efficacious in treating human intestinal parasites.
<b>Adverse events</b>	None reported.
<b>Plant used in food supplement</b>	<i>Cymbopogon citratus</i> (DC.) Stapf ( <i>Andropogon citratus</i> DC., Lemongrass) and <i>Citrus limon</i> ( <i>Citrus limonum</i> , Lemon)
<b>Study design (Quality score)</b>	Randomized control (3)
<b>Study Population and Inclusion Criteria</b>	90 participants : 30 given lemon juice, 30 given lemon grass infusion, 30 given gentian violet aqueous solution 0.5%. Inclusion criteria: A positive diagnosis of oral thrush, currently not on any medication for oral thrush, HIV-positive
<b>Intervention</b>	11 days study period. Treatment group one given 30ml diluted lemon juice, then 2–3 drops of pure lemon juice. Treatment group two given 125ml lemon grass infusion, then 250ml.
<b>Control</b>	30 participants given gentian violet aqueous solution 0.5%
<b>Outcomes measures</b>	Oral thrush scale used, heart rate and blood pressure monitored.
<b>Significance</b>	The lemon juice group showed better results than the gentian violet aqueous solution 0.5% in

	the treatment of oral thrush in an HIV-positive population. The study population was too small to show statistical significance.
<b>Adverse events</b>	The lemon juice group reported changed taste in the mouth and abdominal cramps issues. The lemon grass group reported increased appetite. The gentian violet group reported purple discolouration, cracked lips and dry mouth.
<b>Plant used in food supplement</b>	<i>Trapanatans</i> L. (Water chestnut)
<b>Study design (Quality score)</b>	Open-label observation (0)
<b>Study Population and Inclusion Criteria</b>	5 participants in the preliminary trial. 28 participants in the study with and without treatment: 15 patients with herpes genitalis, 13 patients with <i>Herpes labialis</i> .  Criteria: Recurrent outbreaks of herpes that occurred during compromised general health conditions such as stress, overwork, menstruation, or fatigue, which were not severe enough to interrupt normal everyday life, previous regular outpatient treatment at another medical institution had not been effective in reducing the frequency of outbreaks, cessation of any treatment for herpes at least 2 months before presentation at the clinic, desire to be treated only with the herbal mixture for the present herpes outbreak.
<b>Intervention</b>	6.6 g of herbal mixture containing <i>Wisteria floribunda</i> , <i>Trapanatans</i> , <i>Terminalia chebulae</i> , <i>Coicislachryma-jobi</i> , <i>Ganodermalucidum</i> , <i>Elfuingaapplanata</i> was given two times a day over first two days then once a day over the 30 day period for the group with herpes genitalis and over the 20 day period for the group with <i>Herpes labialis</i> .
<b>Control</b>	Each group were their own controls
<b>Outcomes measures</b>	Two-sided Student's t-test, Meares–Stamey test used.
<b>Significance</b>	The herbal mixture, appeared to provide fast, effective relief from the symptoms of recurrent <i>Herpes genitalis</i> and <i>labialis</i> .
<b>Adverse events</b>	None reported.
<b>Plant used in food supplement</b>	<i>Urticadioica</i> L. (Stinging nettle)
<b>Study design (Quality score)</b>	Randomized blind placebo (2)
<b>Study Population and Inclusion Criteria</b>	143 participants: Group A comprised 106 patients and Group B comprised 37 patients.  Criteria: Presence of symptoms related to CBP for at least 3 months, according to the European Association of Urology (EAU) guidelines, and a positive eares–Stamey 4-glass test with first voided urine, midstream urine, prostatic secretion and a VB3 urine culture, which had to be $\geq 10^3$ colony forming units (CFU)/ml of uropathogens
<b>Intervention</b>	Group A was given 600 mg of antibiotic prulifloxacin with prostamev® ( <i>S. Repens</i> and <i>U. Dioica</i> ) and Flogmev® ( <i>Curcuma longa</i> and Quercetin); while Group B received only antibiotic therapy over 14 days. Follow up 1 month and 6 months from the start of therapy.
<b>Control</b>	No control
<b>Outcomes measures</b>	Meares–Stamey test used.
<b>Significance</b>	Significant differences were found between groups in terms of symptoms and quality of life.  The efficacy of prulifloxacin in patients affected by chronic bacterial prostatitis was improved .
<b>Adverse events</b>	None reported.
<b>Plant used in food supplement</b>	<i>Ziziphuszizyphus</i> (L.) Meikle (jube)
<b>Study design (Quality score)</b>	Open-labeled, (1)
<b>Study Population and</b>	18 participants (Female: 12 Male: 6)

<b>Inclusion Criteria</b>	Mean age (+/- SD) 32.07 (+/- 6.88) years CD4 count of 292 (268.50-338.25) cells/microl
<b>Intervention</b>	90ml Liquid CKBM-A01 containing panax ginseng (1.2% w/v), schisandraechinensis (2.3% w/v) Was taken 2 times a day over a 36-week period. 3 months follow up.
<b>Control</b>	No control
<b>Outcomes measures</b>	Quantification of CD4 cell counts, HIV viral loads, Blood chemistry. Questionnaires
<b>Significance</b>	No significant changes in log viral load or CD4 cell counts were observed at the end of the study. Common colds and nasal symptoms were significantly lower during treatment (p = 0.019). No significant improvement in the treatment of HIV based on CD4 cell counts and viral loads.
<b>Adverse events</b>	Intermittent diarrhea was reported in 55.6%, weakness or skin rash/itching in 50%, and increased bowel movement in 33.7%.
<b>Plant used in food supplement</b>	<i>Prunus armeniaca</i> L. ( <i>Armeniaca vulgaris</i> Lam., Apricot)
<b>Study design (Quality score)</b>	Open study (1)
<b>Study Population and Inclusion Criteria</b>	18 participants Criteria: H. Pylori - infected patients who had positive urea breath tests.
<b>Intervention</b>	130 ml juice given twice a day.
<b>Control</b>	No control
<b>Outcomes measures</b>	Antibacterial effect on H. Pylori by urea breath test (UBT). Delta <sup>13</sup> CO <sub>2</sub> value (UBT value) at 20 minutes after ingestion of urea was measured. Infrared laser spectrometer was used for analysis (ubit-IR200®, Otsuka Electronics, Osaka).
<b>Significance</b>	There was no significant change in UBT values. The bacteria were not successfully eradicated with 2- or 12-week ingestion of the juice.
<b>Adverse events</b>	Adverse events resulting in discontinuing the trial occurred in three cases during 12 weeks: one with worsening constipation at 3 days, one with a transient ischemic attack at 3 weeks, and one with pneumonia at 1 month.

Half the studies did not report on adverse effects (Agrawal et al., 1997; Bailey et al., 2007; Shmueli et al., 2007; Gotteland et al., 2008; Okeniyet al., 2007; Hijikata et al., 2007; Cai et al., 2009). One study conducted on cranberry PFS (McMurdo et al., 2005) reported deaths in both the treatment and control groups. Other symptoms included skin redness, itching, and elevated blood glucose level in a known diabetic patient. The study by Davis et al. on garlic PFS reported adverse effects which included abdominal discomfort, nausea and thrombophlebitis. The study by Caporaso et al. on garlic PFS reported symptoms of nausea, diaphoresis, light-headedness, and severe burning sensations in the esophagus and stomach with vomiting, which was observed in patients who were administered larger amounts of the garlic extract. Maek-a-nantawat et al. reported intermittent diarrhea, weakness or skin rash/itching and increased bowel movement in patients given jujube PFS. Nakajima et al. reported constipation, a transient ischemic attack, and pneumonia in patients that were administered the apricot PFS.

Treatment duration varied widely, from as short as 11 days (Wright et al., 2009) to 3 months (Gotteland et al., 2008). Some had follow up studies conducted on participants, the longest being two years after the clinical trial (Bailey et al., 2007).

### 3.4 Methods for Evaluating the Benefits of PFS as Anti-Infectives

Different methods were identified for evaluating the benefits of PFS as anti-infectives according to the infectious agents under investigation (summarised in Table 3). Bioanalytical methods, statistical programmes and

questionnaires were used. The urine dip stick test and the  $^{13}\text{C}$  urea breath test have been used for detecting bacteria, the Meares–Stamey test for detecting viral infectious agents, microscopic examination of the stool for detecting parasites, and analysis of urine, serum, blood plasma or cerebrospinal fluid for detecting different types of fungi. The samples were analysed using either mass spectrometry, gas chromatography, isotope ratio mass spectrometry or infrared laser spectrometry, based on the micro-organisms under investigation and the sensitivity of the instrumentation required. Five studies (Agrawal et al., 1997; Cai et al., 2009; Hijikata et al., 2007; Gotteland et al., 2008; Wright et al., 2009) reported statistical significance in benefit claims of the PFS on specific outcomes against the different infectious diseases.

Table 3. List of methods identified in this review for detecting infectious agents for evaluating the benefits of PFS

Method	References	Infectious agents measured	Advantages	Disadvantages
Urine dip stick test	Bailey, 2007, Mcmurdo 2005	<i>Helicobacter pylori</i> bacteria	Quick and inexpensive method	Non-quantitative
$^{13}\text{C}$ urea breath test	Shmuelly, 2007, Gotteland, 2008, Nakajima, 2006	<i>Helicobacter pylori</i> bacteria	Non-invasive, quick method	Non-quantitative
Meares–Stamey test	Hijikata, 2007, Cai, 2009	Viral infectious agents such as Herpes	evaluate localised pathogens	None
Microscopy of the stool	Agrawal, 1997; Okeniyi, 2007; Bailey, 2007	Intestinal parasites, bacteria	Simple, inexpensive, quantitative method	None
Analysis of urine and serum	Caporaso, 1983	cryptococcus fungi	Convenient, fast, quantitative method	None
Analysis of blood plasma and cerebrospinal fluid	Davis, 1990	fungi causing cryptococcal meningitis	quantitative	Invasive method
Oral thrush scale	Wright, 2009	oral thrush fungi	Quick	Not quantitative

#### 4. Discussion

Based on the search strategy conducted in this review not many plant species that are used in food supplements in Europe have been clinically evaluated for anti-infectious efficacy in humans. This can be explained by the regulations imposed on PFS, which state that they cannot make any medical claims such as the prevention and treatment of a disease (FDA). Should these plant species have efficacy in treating infectious diseases, they should be registered as complimentary or alternative natural medicines. PFS can however have claims associated with improving the immune system and relieving symptoms of infectious diseases.

According to the European Food Safety Authority (EFSA) many botanical species are used in both food supplements and traditional medicinal products which belong to different regulatory domains with a broad overlap (Silano et al., 2011). The Food Supplements Directive (FSD) Directive 2002/46/EC, established a definition for food supplements as foodstuffs, the purpose of which is to supplement the normal diet and which are concentrated sources of nutrients or other substances with a nutritional or physiological function, alone or in combination, marketed in dose form, namely forms such as capsules, pastilles, tablets, pills and other similar forms, sachets of powder, ampoules of liquids, drop dispensing bottles, and other similar forms of liquids and powders designed to be taken in measured small unit quantities. Botanical food supplements are intended to complement the normal diet, whereas medicinal products should be mainly intended for treating or preventing specific symptoms of disease. Herbal products may be classified and placed on the market as food provided that they do not fulfill the definition of medicinal products and that they comply with the applicable food law. In particular, herbal products marketed in the form of food supplements should comply with Directive 2002/46/EC on food supplements and Regulation 1924/2006 on nutrition and health claims made on foods. Health claims regarding botanicals such as disease risk reduction claims are considered on a case-by-case basis by the EFSA.

In this review we found that only 19 of the 309 plant species were clinically evaluated for prevention and/or treatment of infectious diseases. A total of 13 clinical trials (reported in English) were reviewed to evaluate the experimental approaches used to support the benefit claims of plant food supplements (PFS) associated with infectious diseases.

While the current review only reports the evaluation of the 10 plant species in FS, we identified and evaluated the suitability of the methods most commonly used in evaluating PFS effect on the different infectious agents.

The overall quality of the clinical studies in this review varied, especially in design where some studies were blinded versus open and some studies had control groups to compare the efficacy of PFS with and without the active plant ingredient/s under investigation.

The majority of clinical trials under review investigated single ingredient formulated supplements in capsule, liquid, or injectable form, half of which were sourced from commercial suppliers. A few studies investigated PFS that consisted of a mixture of ingredients as the treatment regime and the benefit claim cannot be made on the single plant species under investigation in this review for each particular study (Agrawal et al., 1997; Hijikata et al., 2007; Caiet al., 2009; Maek-a-nantawat et al., 2009). Some studies investigated the additive effect of the PFS in patients treated with current drugs (Gotteland et al., 2008; Caiet al., 2009; Shmuely et al., 2007). Wright et al. investigated lemon juice versus lemon grass to evaluate the antifungal effect of the two PFS.

A range of study designs were employed across the trials. Blinded randomized placebo controlled and double blind placebo trials were used to evaluate the antiparasitic effects of PFS; open and randomised, controlled trials were used to evaluate the antifungal effects of PFS; and open-label trials were used to evaluate the antiviral effects of specific PFS. The antibacterial effects of PFS were evaluated using a variety of study designs such as open label, blind randomized placebo controlled, double blind randomized placebo controlled, multicentric double blind randomized placebo controlled and prospective double blind placebo controlled crossover trials.

Interestingly studies with small populations showed significant benefits of the use of PFS to treat symptoms associated with infectious diseases. However, small groups of participants are not a true representation of the general population which have a combination of different ailments at any given time in their lifespan. At the same time, small groups often lack the sample power required to detect a difference that is real.

Only half of the 13 studies reported on adverse effects which were quite severe in some cases including death in one of the studies. Some of the PFS may cause relief of the symptoms being treated, however, they may cause other undesirable adverse effects which may be as problematic as the symptoms being treated. This is a major concern associated with the use of PFS, and it is essential that clinical safety studies be conducted first to determine the safe dose of the PFS before evaluating the efficacy. It is recommended that all PFS that do not have clinical safety data or history of safe use should be taken off the market. Also, stringent quality control and quality assurance should ensure the availability of high quality, standardised PFS, containing a specific percentage of marker/active compounds.

A broad range of bioanalytical methods were identified in this review for detecting the different infectious agents. Analysis of urine, serum, blood plasma or cerebrospinal fluid were methods employed for detecting different types of fungi. Urine analysis using a dipstick and/or <sup>13</sup>C urea breath test were used for detecting bacteria, while microscopic examination of the stool was used to measure both bacteria and parasites. The Meares–Stamey test was used to measure viral content. Diary cards and questionnaires were also used for patients to monitor the benefit or adverse effects arising from the studies. These are valid methods for qualitative and quantitative analysis (Devillé et al., 2004; Gill et al., 2003; Savarinoa et al., 1999; Zegarra et al., 2008).

The number of articles would not allow for significant conclusions since overall only 27 articles representing 19 plant species were identified for having been tested against the different types of infectious diseases.

Due to the limited number of studies conducted on PFS as anti-infectives, open trials were included (which represented approximately 50% (6) of the total (13)) were included in this review. The six studies with the open trial design were not limited to a particular infection i.e. bacteria, fungi or virus.

## 5. Conclusions

Although the objective of this review was to evaluate the experimental approaches used in clinical trials to support the benefit claims of plant food supplements (PFS) with reported activity on infectious diseases, the medicinal claims of the PFS should also be considered to assist the regulatory bodies.

The existing clinical trials have the methodological soundness to identify anti-infectious properties of the plant food supplements. However, the plant ingredients within the food supplements should be standardised according to dose and dosage form to substantiate the safety and effectiveness in PFS.

The EFSA and EMA should collaborate to help the European Commission to clearly establish differences on indications of use for food supplements and medicinal products.

Regulatory bodies should advise on design specifications to accept registration of plant food supplements which are different from medicines. This would result in standardisation of the process and reporting regarding clinical studies.

The experimental approaches used were found to be suitable for analysing biomarkers, or symptoms associated with treating infectious diseases (Devillé et al., 2004; Gill et al., 2003; Savarinoa et al., 1999; Zegarra et al., 2008), therefore benefit claims of PFS can be made for treating symptoms of the infectious diseases. The patient sample size and the overall quality of the study design appeared to play a bigger role in influencing the outcome of the trials. Small groups often lack the sample power required to detect a difference that is real. The use of more sensitive instrumentation for analysis will provide better statistical significance between the treatment and control groups. The PFS cannot be used to alleviate the disease itself as stipulated by the regulations.

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