Acne vulgaris is a multifactorial chronic disorder of the skin pilo-sebaceous follicles. Its pathogenesis is not completely understood, although Propionibacterium acnes, the dominant microbe in the sebaceous glandular regions, and inflammation, possibly initiated by this bacterium, appear to be the two main instigators and drivers of the disease. In addition, other important contributing factors include several hormones and host nutritional status (Kurokawa et al., 2009; Lyte et al., 2009). One explanation for the chronic nature of the disease is that P. acnes induces the production of pro-inflammatory cytokines and chemokines, as well as other inflammatory mediators, which attract leukocytes to the site of infection and thereby set up a cascade of inflammatory responses, which also involve the production of reactive oxygen species and other radicals, all of which in combination lead to the development of the acne lesion (Docherty et al., 2007; Kurokawa et al., 2009).

Conventional therapy has targeted the development of the lesions, by means of retinoic acid analogues and other compounds, and antibiotics directed against the bacterium (Docherty et al., 2007). Needless to say, the continued application of antibiotics entails the risk of resistant bacteria emerging. As an alternative approach, several recent reports have indicated the possibility of using plant extracts to counter the growth of the bacteria and/or the inflammatory response, although these have yet to be evaluated in vivo (Chomnawang et al., 2007; Joo et al., 2008; Kim et al., 2008).

Another prospective safe alternative is the use of standardized preparations of Echinacea species, which are currently very popular herbal remedies for ‘colds and flu’, although in traditional medicine in North America Echinacea preparations were also advocated for the treatment of skin lesions, especially for wound healing (Barrett, 2003; Barnes et al., 2005). More recent monographs have suggested the topical use of Echinacea purpurea in semi-solid or liquid form for cutaneous use and the treatment of small superficial wounds (HMPC Monograph, 2008). This suggests possible value as an antibacterial and antiinflammatory agent. However, there is considerable variation in the bioactivities of preparations derived from different species and parts of the plant (Hudson et al., 2005; Vohra et al., 2009). It was reported recently that a commercial standardized preparation of Echinacea purpurea (Echinaforce®) possesses efficient and selective antiviral and antibacterial properties, in addition to antiinflammatory properties, with no cytotoxic effects (Sharma et al., 2009a). Therefore it was decided to evaluate Echinaforce® for its ability to control or inactivate P. acnes, and to inhibit bacterial induction of pro-inflammatory cytokines, as determined by cytokine antibody arrays. The advantage of array technology is that multiple cytokines and chemokines can be analysed simultaneously, and this formed the basis of our recent study on virus-induced pro-inflammatory responses and their reversal by Echinaforce® (Sharma et al., 2009a).

INTRODUCTION

Acne vulgaris is a chronic inflammatory disorder of skin follicles caused by the gram-positive bacterium Propionibacterium acnes. The possibility was investigated that a standardized preparation of Echinacea purpurea (Echinaforce®), with known antiviral, antiinflammatory and antibacterial properties, might provide a useful alternative treatment in the control of the disease. The herbal extract readily killed a standard laboratory strain of the bacterium and several clinical isolates. In cell culture models of human bronchial epithelial cells and skin fibroblasts, P. acnes induced the secretion of substantial amounts of several pro-inflammatory cytokines, including IL-6 and IL-8 (CXCL8), as determined by means of cytokine–antibody arrays. However, the E. purpurea completely reversed this effect and brought the cytokine levels back to normal. Thus Echinaforce® could provide a safe two-fold benefit to acne individuals by inhibiting proliferation of the organism and reversing the bacterial-induced inflammation. Copyright © 2010 John Wiley & Sons, Ltd.

**Keywords:** Echinacea; acne; Propionibacterium acnes; antiinflammatory; antibacterial; cytokines.
aerial parts of *Echinacea purpurea* (L.) (drug extract ratio 1:12) Moench supplemented with 5% *E. purpurea* roots (drug extract ratio 1:11). HPLC analysis documented the presence of caftaric acid, 264.4 μg/mL; chlorogenic acid, 40.2 μg/mL; cichoric acid, 313.8 μg/mL; echinacoside, 6.9 μg/mL; PIP 8/9 (alkylamide), 36.3 μg/mL; but no caffeic acid, cynarin or polysaccharides (Sharma et al., 2009a). The recommended oral dose is approximately 1.0 mL Echinaforce in 10.0 mL water, corresponding to a final concentration of 1.6 mg/mL dry mass per volume and 6.5% ethanol v/v. No endotoxin was detected (<0.1 unit per mL), according to the Lonza endotoxin test kit (Lonza, Walkersville, MD).

**Antibacterial assays.** The standard ATCC (American Type Culture Collection) strain of *Propionibacterium acnes* was obtained from PML Microbiologics (Wilsonville Oregon). Seven clinical isolates of *P. acnes*, identified in routine blood cultures, were obtained from Dr Diane Roscoe (Microbiology, Vancouver General Hospital). They were all propagated and assayed on sheep blood agar plates, in anaerobic chambers at 35°C. All culture plates and related reagents were obtained from PML Microbiologics (Oregon, USA).

The standard assay method was as follows. Several isolated colonies of each strain were removed and dispersed into PBS (phosphate buffered saline) by vortex mixing, to give a homogeneous suspension of approximately 1 × 10⁸ cfu (colony forming units) per mL. Aliquots of suspension were mixed 1:1 with the diluted extract to give final concentrations of Echinaforce® 1:100 (in PBS), equivalent to 160 μg/mL dry mass per vol plus 0.65% ethanol, or PBS alone, or 0.65% ethanol in PBS, in transparent sterile plastic tubes in ambient light or covered in aluminium foil (for dark exposure), for a period of 60 min with incubation at room temperature (20°C) on a rocker platform. The rationale for comparing light and dark exposure was based on evidence indicating the existence of photoactive ingredients in *Echinacea* extracts (Hudson et al., 2005).

Following the treatment, each mixture was serially diluted (10 × dilutions) in PBS, and replicate 2.5 μL aliquots were spotted onto blood agar plates, divided into sectors for each dilution, and spread uniformly with sterile plastic loops, allowed to dry, and the plates incubated under the conditions described above. After incubation of the plates for 72 h, colonies were counted manually and compared with untreated samples.

**Cytokine analysis.** BEAS-2B cells, a tracheo-bronchial epithelial cell line, and A-549 cells, a lung epithelial cell line, were obtained from ATCC. Human skin fibroblasts (courtesy Dr Aziz Ghahary, Prostate Research Centre, Vancouver) were used in their sixth passage. They were all propagated in Dulbecco MEM (Invitrogen, without antibiotics or antimycotic agents. These were carried out according to the instructions supplied by the companies (R&D Systems, Minneapolis, for IL-8 and TNFα, or e-Bioscience, San Diego, CA, for IL-6).

**Cytokine antibody arrays.** The Raybiotech fluorescent antibody array system was used. The array format (QAH-CYT-1 from Raybiotech Inc. Norcross GA) consisted of quadruplicate antibody spots for 20 cytokines and inflammation-related mediators. The array slides were incubated with cell-free supernatants, and processed according to the manufacturer’s instructions. Data acquisition was performed via a Perkin Elmer ScanArray Express laser microarray scanner (courtesy the Vancouver Prostate Centre Microarray Facility) and subsequent quantification using ImaGene 8.0 software from BioDiscovery. Signal intensity medians were background corrected and the means and standard deviations of the replicates calculated. Some of the slide wells were treated with pure antigens (as part of the Raybiotech fluorescent antibody array system kit) in order to calculate a standard curve. The standard curve was used to convert the calculated mean intensities to concentrations (pg/mL).

**RESULTS**

The *Echinacea* extract (Echinaforce®) showed potent antibacterial activity against the ATCC and clinical strains of *Propionibacterium acnes*, consistently reducing the cfu titre by more than 4 log₁₀. Figure 1 shows the
POTENTIAL USE OF ECHINACEA IN ACNE

Table 1. IL-6 & IL-8 secretion in BEAS-2B cells

<table>
<thead>
<tr>
<th>Supernatant</th>
<th>IL-6 pg/mL</th>
<th>IL-8 pg/mL</th>
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<tr>
<td>Control</td>
<td>10.8 ± 2.26</td>
<td>209.5 ± 4.95</td>
</tr>
<tr>
<td>Control + E (Echinaforce®)</td>
<td>31.8 ± 25.17</td>
<td>299.0 ± 1.41</td>
</tr>
<tr>
<td>P. acnes ATCC</td>
<td>302.6 ± 35.36</td>
<td>1652 ± 4.95</td>
</tr>
<tr>
<td>P. acnes ATCC + E</td>
<td>16.4 ± 0</td>
<td>41.0 ± 0</td>
</tr>
<tr>
<td>P. acnes clinical 1</td>
<td>172.4 ± 14.14</td>
<td>1671 ± 32.53</td>
</tr>
<tr>
<td>P. acnes clinical 1+ E</td>
<td>18.4 ± 2.83</td>
<td>42.5 ± 2.12</td>
</tr>
<tr>
<td>P. acnes clinical 2</td>
<td>283.6 ± 6.22</td>
<td>1688 ± 47.83</td>
</tr>
<tr>
<td>P. acnes clinical 2+ E</td>
<td>53.20 ± 3.39</td>
<td>42.5 ± 2.12</td>
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</table>

DISCUSSION

The objective of the present study was to evaluate a standardized Echinacea extract (Echinaforce®) for its ability to inactivate P. acnes and to inhibit the possible pro-inflammatory effect of this organism. According to our general understanding of acne pathogenesis these are the two main factors that initiate and perpetuate the disease, although other hormonal and nutritional factors play important roles (Kurokawa et al., 2009). The organism itself was readily inactivated even by dilutions of extract well below the normal recommended dose for topical treatment or for oral consumption in the control of colds and flu symptoms.

Several respiratory and skin bacteria were reported previously to be vulnerable to Echinacea extracts (Sharma et al., 2008b), although this property was not common to all types of extract tested, and not all bacteria were equally susceptible. However, Echinaforce® is a standardized preparation that is consistently active against P. acnes. Furthermore, the bacterial induction of pro-inflammatory cytokines, evident in all three cell lines examined, was also inhibited by Echinaforce®, which suggests that this extract could offer dual benefits to acne patients. The bacterial-induced cytokines included IL-6 and IL-8 (CXCL8), and to a lesser extent TNF-α, which are hallmarks of inflammatory responses, and would be expected to lead to an influx of various inflammatory leukocytes. In addition, the secretion of GROα normally results in the attraction of monocytes. Such a combination of cytokines could well explain the production of inflammation at the site of the infection; consequently an agent capable of safely reversing this effect should be beneficial to the patient.

Other plant extracts have recently been reported with anti-Propioni bacteria properties, e.g. Garcinia mangostana (Chomnawang et al., 2007), Selaginella involvans (Joo et al., 2008) and several Korean extracts (Kim et al., 2008), in addition to the isolated phytochemical resveratrol (Docherty et al., 2007). The advantage of Echinaforce® is that it is a standardized and chemically characterized commercial preparation that has been licensed for oral administration, and therefore should be safe for skin applications (Sharma et al., 2009b).

Several other useful properties have been attributed to various Echinacea preparations, including antiviral activities, immune modulating actions, antioxidant activities (Barrett, 2003; Barnes et al., 2005; Sharma et al., 2009b), which could help to control the free radicals associated with acne, and wound healing and for which Echinacea has been described as useful (Rousseau et al., 2006). The latter properties could be impor-
tant in skin applications of *Echinacea*, although they have not yet been described for Echinaforce® per se, which was designed primarily for oral consumption. Cytotoxicity is not a factor in Echinaforce applications (Sharma et al., 2008a, 2009a); and clinical trials have not revealed any side effects (Schoop et al., 2006); consequently safety is not a concern in its use. Therefore we believe that trials of Echinaforce® for the treatment of acne would be worthwhile.

The active ingredients responsible for these activities have not been identified. Polysaccharides, caffeic acid derivatives and alkylamides, common but not universal constituents of *Echinacea* extracts, have been incriminated in various biological effects, but evidence for any of them individually as the ‘active ingredient’ is inadequate (Vimalanathan et al., 2009). In addition, polyynes, which have known antibacterial properties (Deng et al., 2008), are often found in *Echinacea* extracts. It is conceivable therefore that a combination of bio-active compounds, including polyynes, flavonoids or other phenolic compounds, is required.

In conclusion, we believe that the combination of antibacterial and antiinflammatory properties shown by certain *Echinacea* preparations, especially the standardized Echinaforce®, could make this herb a useful adjunct treatment for acne.

### REFERENCES


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<table>
<thead>
<tr>
<th></th>
<th>IL-4</th>
<th>IL-6</th>
<th>IL-8</th>
<th>MCP-1</th>
<th>VEGF</th>
<th>TNF-α</th>
</tr>
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<tbody>
<tr>
<td><strong>C</strong></td>
<td>92.8 ± 24.2</td>
<td>13.5 ± 20.9</td>
<td>9.84 ± 11.9</td>
<td>67.68 ± 22.32</td>
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<tr>
<td><strong>C + E</strong></td>
<td>80.23 ± 24</td>
<td>10.67 ± 8.77</td>
<td>9.2 ± 10.2</td>
<td>54.98 ± 14</td>
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<tr>
<td><em>P. acnes</em></td>
<td>75.86 ± 10.86</td>
<td>94.84 ± 42.7</td>
<td>29.59 ± 8.9</td>
<td>51.5 ± 18.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. acnes + E</em></td>
<td>70.74 ± 25.78</td>
<td>35.39 ± 24.8</td>
<td>3.39 ± 2.4</td>
<td>48.37 ± 15.75</td>
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</table>

**Table 2.** Cytokine antibody arrays of supernatants from cells infected with *P. acnes* ± Echinaforce®. Replicate BEAS-2B monolayer cultures were infected with *Propionibacterium acnes* (ATCC strain, 70 cfu/cell) for 1 h, followed by Echinaforce® (E) at 1:100 dilution. Control uninfected cells were also treated, or not, with Echinaforce®. At 48 h after infection, cell-free supernatants were harvested for analysis by cytokine antibody arrays (as described in Materials and Methods). The fluorescent quadruplicate spots of each cytokine are shown. Pos and Neg spots represent internal positive and negative controls. The relative intensities of the reactions are indicated by colour, with red being the highest reaction and blue the least. Intensity values were converted to cytokine concentrations, in pg/mL, by means of built-in standard curves (not shown) and these values are displayed below the slides for the most significant responders. The arrays for control uninfected cells, C, were indistinguishable from *P. acnes + E*. The significant responders were IL-6, IL-8 (chemokine CXCL8) and GROα, which were all inhibited by Echinaforce®. In addition the normal unstimulated levels of MCP-1, TNFα and VEGF were decreased by *Echinacea*. 


