The traditional medicinal use of *Echinacea angustifolia* can be traced back to the ethnopharmacology of Native Americans who employed the plants for many purposes that relate to the addressing the natural inflammatory response function.\(^1\)\(^-\)\(^3\) Echinacea was also a popular natural remedy in the United States, and was included in the National Formulary of the United States from 1916 to 1950. Notwithstanding the growing use of synthetic drugs, the use of *Echinacea* was rekindled by European studies in the second half of the past century, and *Echinacea* has become one of the most popular dietary supplements.

According to the National Institute of Allergy and Infectious Diseases, the US population experiences 1 billion respiratory challenges every year, and *Echinacea*, known for its immunostimulatory effects, is the most common nutraceutical consumed in the US to address these challenges.\(^4\)

A number of mixed clinical data are available in literature on products obtained from different species of *Echinacea*, using different plant parts and solvents. The indications for these products are generally to support and maintain a healthy immune system.

The available clinical information has been recently reviewed by Barret,\(^5\) who concluded that the globality of the data supports the use of *Echinacea* in the treatment of respiratory challenges, reflecting the most widespread utilization. However, research on efficacy has produced also mixed results.\(^6\)

These mixed findings are not surprising, since the term “*Echinacea*-based preparations” encompasses extracts obtained using varying extraction methods and solvents, from different *Echinacea* species, and from different parts of these plants (e.g., aerial versus underground parts), with marked differences in terms of constituent profiles.

Polinacea\(^®\), extracted exclusively from the roots of *E. angustifolia*, is an immnomodulating standardized extract, studied and patented by Indena.
Two recent preliminary clinical studies conducted at a time of seasonal change, have evaluated the efficacy of Polinacea® to support respiratory health.

The first pilot study enrolled 38 adults. The volunteers were sorted out into three groups, receiving a conventional allopathic intervention, group Polinacea®, or a combination of conventional allopathic intervention Polinacea®, respectively. Polinacea® was administered orally at the dosage of 200 mg/day for the first 15 days of the study, and the dosage was next reduced to 100 mg/die for the following 15 days and to 100 mg/die on alternative days for the remaining 60 days. The administration of Polinacea® showed improvements on the overall subject conditions, and the volunteers who received the combination of Polinacea® and conventional allopathic intervention experienced the least respiratory challenge during the time frame of the study.

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Challenge-like Symptoms</th>
<th>Semi-challenge-like Symptoms</th>
<th>Challenge Mild</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional allopathic intervention Group (14)</td>
<td>0</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Polinacea® Group (12)</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Conventional allopathic intervention + Polinacea® Group (12)</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Effect of oral administration of Polinacea® on adult subjects with existing respiratory challenges.

The second pilot study enrolled 34 healthy pediatric subjects (whose participation was authorized by their parents and overseen by an Ethical Committee in accordance with Good Clinical Practice guidelines), who received Polinacea® (100 mg/die for the first 30 days, then reduced to 100 mg/die every other day for the following 60 days) or a B vitamin supplement. None of the participants received conventional allopathic intervention, and at the end of the three-month study, a reduction in the occurrence of respiratory tract challenges was observed in the Polinacea® branch.

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Challenge-like Symptoms</th>
<th>Semi-challenge-like Symptoms</th>
<th>Challenge Mild</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polinacea® Group (14)</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Vitamin Complex (20)</td>
<td>2</td>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>

Effect of oral administration of Polinacea® on healthy subjects.

A new pilot study involving 10 human healthy volunteers has provided a putative mechanistic basis. After four weeks of administration of 100 mg Polinacea® as a syrup, genomic analysis (mRNA levels in lympho- and monocytes) evidenced a upregulation of the production of the cytokines IL-2 and IL-8, associated to downregulation of the production of IL-6 and TNF-α cytokines. Plasma measurements confirmed these changes, except for TNF-α, whose levels were not significantly changed. These results further support the concept that Polinacea® can modulate cytokine expression in humans, positively supporting respiratory health.
Polinacea® potential as an immune response enhancer is based on the results obtained in several in vitro models. In order to avoid an unspecific response of the immune competent cells for the in vitro studies, samples of Polinacea® were purified from lipopolysaccharides (LPS) of bacterial origin, which are a possible contaminant of the plant root utilized for the extraction. LPS, in fact, are reported to produce a non specific immune response on macrophages in vitro.

In immunocompetent mice challenged with Leishmania major (one of the most representative experimental animal models utilized to ascertain the immunoboosting capability of substances), Polinacea® reduced by over 25% the experimentally induced leishmaniasis (mortality at week 1). Moreover, Polinacea® orally administered at the dose of 1g/kg day for 7 days was effective (30%) in counteracting the mortality induced by Candida albicans in immunocompetent mice; even in the case of Cyclosporin-immunosuppressed mice, Polinacea® was able to prevent animal death by 40%.

In terms of mechanism of action, there are animal models to confirm the hypothesis of a direct action on T cells: Polinacea® and IDN 5405 have been both deprived of LPS (responsible of non specific immune response). They have been shown to dose dependently stimulate anti-CD3-treated isolated T limphocytes to produce and release interferon-g (IFN-g). Anti-CD3 are reported to affect immune responses by inducing immune regulation.[9] These results have been paralleled by a good response in terms of cell proliferation of T lymphocytes. In the same animal model, a reference product (selling well-established European Echinacea), compared to a placebo, has not given statistically significant results.

### Treatment | n | Survivors
--- | --- | ---
Candida albicans | 10 | 0
Candida albicans + Polinacea® (1g/kg day x 7 days) | 20 | 6*
Candida albicans + Cyclosporin A (1g/kg day x 7 days) | 10 | 0
Candida albicans + Cyclosporin A + Polinacea® (1g/kg day x 7 days) | 10 | 4*

Effect of oral administration of Polinacea® on survival in normal and immunosuppressed mice infected with Candida albicans.

* p< 0.01 vs control

Polinacea® intraperitoneally administered at the dose of 0.1g/kg day was effective in counteracting mortality induced by Candida albicans. This effect was also supported by the fact that LPS (lipopolysaccharides), administered at a dose of 2 μg/kg day, corresponding to the amount administered in LPS-containing Polinacea®, was practically ineffective.
Polinacea® is a standardized extract from the roots of a wild *E. angustifolia* variety, selected and cultivated by Indena. The extract is standardized in echinacoside (≥2%), and a structurally unique high molecular weight polysaccharide characterized by the presence of a partially carboxymethylated and partially acetylated polygalacturonic acids, with accompanying rhamnogalacturonan (≥5%), named IDN 5405.

The occurrence of this polysaccharide in the roots of *E. angustifolia* was first reported by an Indena research group. IDN 5405 is a polysaccharide that has been highlighted for the first time in the root of *E. angustifolia*.

Polinacea® has a unique triple standardization in the following constituents: echinacoside, a caffeoylated polyphenol of the phenylethanoid class; IDN 5405, a high molecular weight polysaccharide of ca. 20,000 Da, identified for the very first time in the *E. angustifolia*; Polinacea® is also standardized for being devoid of alkamides (isobutylamides) (≤0.1%). Isobutylamides have a powerful inflammatory response support function, mediated by the activation of the peripheral cannabinoid receptor. Consequently, consumption of isobutylamiderich *Echinacea* preparations may contribute to supporting a healthy inflammatory response during an challenge in progress, but might not exert any immuno-stimulating or overall effect on morbidity. When this information is combined with their chemical instability and the current limited knowledge of their toxicity, it provides a rationale for removing alkamides from *Echinacea* extracts intended to be used exclusively for immuno-stimulating purposes. Thus, while Polinacea® is more indicated for the prevention of respiratory challenges, *Echinacea* preparations rich in isobutylamides might be more useful to alleviate the symptoms of this condition.

### Chemical profile

<table>
<thead>
<tr>
<th>Standardized compound</th>
<th>Chemical nature</th>
<th>Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Echinacoside</td>
<td>Caffeic acid derivative</td>
<td>≥ 2%</td>
</tr>
<tr>
<td>IDN 5405</td>
<td>High molecular weight polysaccharide</td>
<td>≥ 5%</td>
</tr>
<tr>
<td>Isobutylamides</td>
<td>Amides</td>
<td>≤ 0.1%</td>
</tr>
</tbody>
</table>

Triple standardization of Polinacea®.
**Botanical profile**

*Echinacea angustifolia* DC (narrow leaved-purple coneflower) is one of the “coneflowers”, a group of Native American wildflowers from Asteraceae family characterized by spiny flowering heads and with an elevated receptacle which forms the “cone”. The species is a herbaceous perennial, and flowers late in Spring-mid Summer, forming vertical taproots in dry prairies, barrens, rocky sandy soil from Texas to Saskatchewan, and from Western Iowa to Minnesota.[13,14] The ethnopharmacology of Native Americans underlies most of our knowledge on North American plants, and *Echinacea* spp. represent the most relevant example.[14]

Samples of *Echinacea* spp. have been found in archeological digs of Lakota Sioux village sites from 1600s and most of the information we have on the ethnobotany of *Echinacea* spp. comes from Native American tribes. For traditional medicinal purpose and ornamental purposes, three different species are cultivated: *Echinacea angustifolia* DC, *E. pallida* (Nutt.) Nutt. and *E. purpurea* (L.) Moench. The first two species are often confused; in particular the more abundant and easily cultivated *E. pallida* is traded under the name of *E. angustifolia*, a species better documented in terms of biological activity.[16]

Indena’s Quality Control by morphological and chemical examination can distinguish these two species. Furthermore, in order to improve the consistency and quality of its product, Indena has established dedicated plantations to provide its supply of *E. angustifolia*.

**Conclusive remarks**

The in vivo studies conducted on Polinacea® revealed its immune boosting capacity despite the presence of LPS activating macrophages.

The positive results on the in vitro T cells suggest Polinacea® is effective when employed as immunostimulant. Its action is seemingly related to the combined action of an immunostimulating polysaccharide (IDN 5405) and the properties of the polyphenolic echinacoside.

**A few milestones about Polinacea®:**
- selected cultivated *Echinacea angustifolia* plants
- innovative extraction procedure
- unique triple standardization
- low level of isobutylamides
- direct immunological effect on T cells
- well tolerated in acute and subacute toxicity
- shown effective in conjunction with conventional allopathic intervention in two pilot clinical studies.

**Safety profile**

The studies conducted on Polinacea® (oral acute and subacute toxicity) indicate that the product has no toxic effect at all tested dosages. No signs of any clear toxicological effect were seen in the subacute toxicity at any of the dose levels investigated (100, 300 and 1000 mg/kg/day) and thus, the dose of 1g/kg/day may be considered the “no observed adverse effect level” – NOAEL, based on these studies.