“Method for Extracting Active Ingredient of ECHINACEA”

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Abstract: Echinacea Purpurea (Asteraceae) is a perennial medicinal herb with important immunostimulatory and anti-inflammatory properties. Especially the alleviation of cold symptoms. The plant also attracted scientist’s attention asses other aspects of it’s beneficial effects. For instance, antianxiety, antidepression, cytotoxicity and antimutagenecity induced by the plant have been revealed in various studies. The findings of the clinical trails are controversial in terms side effects. While some studies revealed beneficial effects of the plants on the patient and no serve adverse affect, some others have reported serious side effects including abdominal pain, dyspnea , nausea, rash, erythema. Other biological activities of the plant such as antioxidant, antibacterial, antiviral have been reported in previous experimental studies. Different classes of secondary metabolites of the plants such as alkaloids, caffeic acid derivatives, polysaccharides and glycoproteins are believed to be biologically and pharmacologically active. Actually concurrent determination and single analysis of cinchoric acid and alkaloids have been successfully developed mainly by using high performance liquid chromatography (HPLC) coupled with different detectors including uv spectrophotometric, coulometric electrochemical and electro spray ionization mass spectrometric detectors. The result of the studies which were controversial revealed that in spite of major experiments successfully accomplished using Echinacea purpurea many questions remain unanswered and future investigations may aim for complete recognition of the plant mechanism of the action using new complemetary methods.

Key Words: Anti-inflammatory, cytotoxic, antibacterial, anti-depresssion, psychotic, etc.

I. INTRODUCTION:

Echinacea purpurea Moench is one of the most important and well known medicinal plants in the world, belonging to the Asteraceae family. The plant is most widely cultivated medicinal plant in the species, which have been mainly used in chemopreventive and chemotherapy for infectious diseases in both upper and lower respiratory systems. The species has been trationally employed for the treatment of toothache, bowel pain, snake bite, skin disorders, seizure, chronic arthritis and cancer. Taxonomic, chemical, pharmacological and clinical characteristics of some species of echinacea genus including Echinacea angustifolia, Echinacea Pallida, Echinacea purpurea were reviewed in previous papers. Medicinal properties of the plant were considered in review paper, which suggested that more research is required for more definitive medicinal recommendations.

Echinacea have been found in archeological dics of lakota sioux village sites from the 1600S and its popularity has increased since it has been known to the european based settlers from the turn of the 19th century. Echinacea plant have different species consist of herbacious perennial plants that are indigenous of North America and have been traditinally used to treat various ailments.

Echinacea is currently used for it’s antioxidant properties, infectious disease, respiratory
tract infections and as an immunostimulant.[8] The common name for Echinacea is purple cone flower and it is characterized by daisy-like head that consists of several tiny flower.[9] Echinacea purpurea, Echinacea angustifolia and Echinacea pallida are three species of Echinacea that are primarily used medicinally, particularly roots and rhizomes of the plants. Also the flowering tops of Echinacea purpurea are used in medicinally.[10]

The commercial products of Echinacea purpurea include tinctures, teas, beverages, tablets, capsules and personal care products.[11] An earlier studies stated that aerial parts of Echinacea purpurea and Echinacea angustifolia root also contain alkamides polysaccharides.[12] In vitro and in vivo studies of Echinacea and Nk cells have been conducted as well. And in vitro study indicated that water soluble extract of Echinacea activated cytotoxicity of NK cells.[13]

This paper is a review about Echinacea purpurea. It’s phytochemical contents and it’s pharmacological and biological activities along with common methods of extract analysis in addition psychoactive and mosquitocidal effect of plant are mentioned in this paper.

2. METHODS:

Alkaloids have been analysed with reverse phase HPLC coupled with different detectors including uv spectrometric coulometric electrochemical, and electrospy ionization mass spectrometric.[14]

Further more, caffeic acid derivatives have been determined using reverse phase HPLC or capillary electrophoresis with photodiode array (EDA) uv spectrophotometric detection.[15] Phenolic acids were analysed by micellar benzoic acid electrokinetic chromatography (MEKC) both charged and uncharged analytes, based on the use of sodium deoxycholate (SDC) a surfactant in borate buffer (PH : 9.2) As well as echinacea purpurea extract.[16] However, determinatoin methods for both caffeic acid derivatives and alkamides have the advantages of reduced time and sample size needed for the analysis.[17]

Simultaneous analysis of both mentioned derivatives has also been performed by electrophoresis with FDA in uv spectrophotometric detector toghether with sodium dodecyl sulphate and hydroxypropyl beta cyclodextrin in Britton Robinson buffer.[18]

3. Extractin of acive compound from Echinacea purpurea flowers.

3.1. Multi-Step Method

The extract was obtained to the methods Tsai et.al with minor modificatoins[19] The fridge dried flower powders (15gm each sample) were extracted with 150 ml of 50% aqueous ethanol in shaking bath at 100 rpm for 30 min under 65 degree celsius conditions and centrifused at 3460 x gm for 10 min filtered advantec no 1 filter paper. The resultant dry extracts were stored at -20 degree celsius before use. all experiment done triplicate.

3.2 DETERMINATIOIN OF PHENOLIC ACIDS.

3.2.1. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Quantitative analysis of phenolic compounds in plant materials depends upon the chemical nature of the constituents, the method of extraction particle size, and storage condition of the plant material prior to analysis, as well as on the determinat method and the presence of interfering agents such as fats, terpenes and chlorophyll.[20]

Liquid chromatography uv detection was also applied for the determination of phenolic acids in Echinacea purpurea.[21] the leaves of lemon balm,[22] aqueous extracts of hypercium perforatum[23] and 32 medicinal plant growing in poland.[24]
Table no. 1: Comparision of LC - MS with GC - MS for the analysis of phenolic compounds in medicinal plants.

<table>
<thead>
<tr>
<th>Sr.no</th>
<th>Parameters</th>
<th>LC-MS</th>
<th>GC-MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Time of sample preparation</td>
<td>20min</td>
<td>180min</td>
</tr>
<tr>
<td>2.</td>
<td>Time of analysis</td>
<td>60min</td>
<td>50min</td>
</tr>
<tr>
<td>3.</td>
<td>Range of linearity</td>
<td>Limited</td>
<td>Good</td>
</tr>
<tr>
<td>4.</td>
<td>Selectivity</td>
<td>Good</td>
<td>High</td>
</tr>
<tr>
<td>5.</td>
<td>Limit of detection</td>
<td>5-15mg/ml</td>
<td>10-80mg/ml</td>
</tr>
<tr>
<td>6.</td>
<td>Ruggedness of system</td>
<td>Satisfactory</td>
<td>Very good</td>
</tr>
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</table>

5. SUPERCRITICAL FLUID EXTRACTION METHOD:

Supercritical fluid extraction is a modern technique, which has many advantages over the classical extraction methods. [25,26] Other advantages are high selectivity, significant reduction in solvent volumes used for extraction, low mass of sample for extraction, short extraction time, possibility of automation, as well as offline and online coupling with majority of chromatographic techniques (GC, HPLC).

SFE is commonly applied in the pharmaceutical Industry, as well as in the food and cosmetic areas. [27] Supercritical fluid extraction serves for the isolation of biologically active compounds from plant materials, mainly for those cannot separated by the use of simple solvent extraction.

4: ACCELERATED SOLVENT EXTRACTION (ASE):

In the ASE technique the same solvents are used those in classical methods, but a higher pressure (about 3.3-20.3MPa) and elevated temperature about 40-200°C are applied. [28]

A sample extracted by this technique is placed in an extraction vessel made of stainless steel. The time of analysis is short, within the range of 5-15min. [29]

6. TOXICOLOGY:

Generally, animal studies of various preparations of Echinacea species have shown low toxicity. [30] In study of acute toxicity, LD 50 value was calculated as 2500 mg/kg in an intraperitonial injection of polysaccharide fraction of the plant in female mice. [31] In other studies the LD 50 values of oral and IV administration of the plant juice evaluated more than 30gm/kg, 10 gm/kg in mice and 15 gm/kg, 5 gm/kg in rat respectively. [32] A polysaccharide fraction isolated from Echinacea purpurea was reported as negative for mutagenicity in genotoxicity human lymphocyte assay. [33] Maximum feasible oral and IV doses of ethanol stabilized fresh pressed juice of Echinacea purpurea have similarly been reported as negative for measurable damage in mice or rate. [34] Injection of Echinacea purpurea extracts into chick embryoes failed to cause detectable changes in development, so far, screens for toxicity have been overwhelmingly negative. [35]

7. CONTRAINDICATION:

The Echinacea preparations are contraindicated in some patients including those with progressive diseases such as tuberculosis, leukemia and leukemia like diseases, collagen disorders and other autoimmune diseases. [36] The preparations of Echinacea should be administered with caution concomitantly with immunosuppressant drugs. [17] Some Echinacea products are also contraindicated in AIDS and HIV infections. These based on the theory of immunomodulatory activity of Echinacea so, there is an apposing idea that these products are not harmful in patients with autoimmune diseases. [38] Effects of some preparations made from root and herb of E. Purpurea along with cichoric acid were tested on the human drug metabolising enzyme cyt. P450 3A4 (CYP3A4). The result indicated that the preparations moderately inhibited the enzyme, while cichoric acid showed low inhibition activity. [39] The constituent of the plant that are responsible for CYP3A4 inhibition are not systematically available the constituents that are responsible for CYP3A4 induction may rapidly be absorbed leading to lack of intestinal CYP3A4 induction, hepatic CYP3A4 induction may occur for by the metabolites of the plant or CYP3A4 induction may involve tissue activates that are influenced by constituents of the plant. [40]

The plant inhibited metabolism of testosterone by CYP3A4 nicotinamide adenine dinucleotide phosphate (NADPH) dependent reaction. [41]
8. DISCUSSION:

Echinacea purpurea has worldwide reputation for its immunomodulatory and anti-inflammatory properties capable of modulating various immune system pathways. There are different classes of secondary metabolites of the plant showing immunostimulatory activity such as alkamides, caffeic acid derivatives, polysaccharides, and glycoproteins. [42] The polar extracts of the plant usually contain more polar metabolites including polysaccharides and glycoproteins. [43] Another compound like cichoric acid, amides have also been isolated from the plants. [44] As alkamides and caffeic acid derivatives have been considered the main and particular secondary metabolites of the plant, different methods have been employed for concurrent or separate analysis of them such as high performance liquid chromatography (HPLC). [45] After review of the available literature all medicinal species of Echinacea including E. Purpurea, E. Angustifolia, and E. Pallida appear to quite safe. While the absence of severe drug-related adverse events does not conclusively prove safety, it is an indication that significant acute toxicologically events are lacking.

In a toxicity study by Menges et al. concluded that even a lethal dose was not found. [46]

9. USES:

9.1 Anti-inflammatory Effects:
The Echinacea preparation interestingly has reversed the inflammation caused by some bacteria in a culture of epithelial cells by reducing cytokines. [47] Dried root powder of the plant, administered to the mice (30-100 mg/kg), inhibited carrageenan-induced paw edema similar to indomethacin. [48] This effect may be attributed to the inhibition of COX 1 and to a lesser extent COX 2 by alkamides. [49]

9.2 Psychoactive Activity:
The anxiolytic activity of Echinacea drugs was determined in experimental animals with lower doses than those used in traditional indication. [50] Plants rich in alkamides induced paresthesia and were applied by Native Americans traditionally and also by physicians in the early 20th century dialogue and antitussive and remedy for toothache. [51]

9.3 Mosquitocidal Property:
Purified alkamides from E. Purpurea show mosquitocidal activity against Aedes aegypti larvae. The alkamides with isobutalamide moiety show stronger mosquitocidal activity compared to those with 2-methyl -butylamide moiety suggesting that isobutyl plays role in the mosquitocidal property of alkamides. [52]

9.4 Anti-androgenic Activity:
The effect of E. purpurea root extract on the weight of the prostate in rats, rat testicle, and epidymis as well as alteration of histological showed the antiandrogenic activity. [53]

9.5 Anti-tumor Activity:
The hexanic root extract of the root was seen to have cytotoxic and proapoptotic properties. It reduced cell viability in concentration and time-dependent manner. This result represented starting point to establish viable scientific evidence on the possible role of echinacea species in medical oncology. [54]

9.6 Radio protective activity:
The activity was assessed on the gamma-irradiated mice which was due to E. Purpurea extract the result reflected the detrimental reduction effects of gamma rays on a peripheral blood haemoglobin and the levels of red blood cells, differential white blood cells, and bone marrow cells. [54]

9.7 Anti-microbial activity:
The methanolic and aqueous extract of E. Purpurea showed resistance to influenza A2, herpes and vesicular stomatitis infection for 24 hrs when incubated on mouse fibro glass and also against Herpes simplex virus and influenza virus a high weight molecular fraction containing polysaccharide and glycoproteins responsible for the activity. [55]

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