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"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, antideppression, cytotoxity 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 alkamides,caffeic acid derivetives, polysaccharides and glycoprotiens are believed to be biologically and pharmacologically active. Actually concurrent determiantion and single analysis of cinchoric acid and alkamides have been successfully developed mainly by using high performance liquid chromatography (HPLC) coupled with differenr detecors including uv spectrophotometric ,coulometric electochemical and electro spray ionization mass spectrometric detecors .The result of the stuidies 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 pupurea 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.[1]The species has been trationally employed for the treatment of toothache,bowel pain ,snake bite,skin disorders,seizure,chronic arthritis and cancer.[2] Taxonomic,chemical,pharmacological and clinical characteristics of some species of echinacea genus including Echinacea angustifolia,Echinacea Pallida,Echinacea purpurea were revieved in previous papers.[3,4] Medicinal properties of the plant were considered in review paper,which suggested thar more research is required for more definctive medicinal recommendations.[5]

.Echinacea have been found in archeological dics of lakota sioux village sites from the 1600S[2] and its popularity has increased since it has been known to the european based settlers from the turn of the 19th century.[6] Echinacea plant have different species consist of herbacious perennial plants that are indigenous of North America and have been traditinally used to trteat various ailments.[7]

Echinacea is currently used for it's antioxident 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 erxtracst 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 psycho active and mosquitocidal effect of plant are mentioned inthis paper.

2.METHODS:

Alkaloids have been analysed with reverse phase HPLC coupled with different detectors including uv spectrometeric coulometric electrochemical, and electrospray 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 benzioc acid electrokinetic chromatography (MEKC) both charged and uncharged analytes, based on the use use of sodium deoxycholate (SDC) a surfactant in borate buffer (PH : 9.2) As well as echinacea purpurea extract .[16] However,determinatoins methods for both caffeic acid derivatives and alkamides have been developed in single analysis. Although it is difficult process to seperate this diverse constituents in one analysis method for the concurrent determinatoin of 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 cycliodextrin 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% aqeous 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 DETERMINATOIN OF PHENOLIC ACIDS .

3.2.1.HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Quantitativeanalysis 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 cholorophyll.[20]

Liquid chromatography uv detection wasalso applied for the determination of phenolic acids in Echinacea purpurea. [21] the leaves of lemon balm .[22], aqeous 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.

Sr.no	Parameters	LC-MS	GC-MS
1.	Time of sample preperation	20min	180min
2.	Time of analysis	60min	50min
3.	Ranege of linearity	Limited	Good
4.	Selectivity	Good	High
5.	Limit of detection	5-15mg/ml	10-80mg/ml
6.	Ruggedness of system	Satisfactry	Very good

5. SUPERCRITICAL FLUID EXTRACTION METHOD:

Supercritical fluid extraction is a modern technique,which has many advantages over the classical extraction methods[25,26] 7

Other advantages are high selectivity,significant reduction in soivent volumes usedZ 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 classiccal methods, but a higher pressure (about 3.3-20.3MPa)and elevated temperature about 40-200^{0}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:

Genrally ,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 and15 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 overuhelmingly negative .[35]

7. *CONTRAINDICATION :*

TheEchinacea prepartions are contraindicated in some patients including those with progressive diseases such as tuberculosis ,leukemia and leukemia like diseases ,collagen disorders and other autoimmune dieseases.[36] The preparations of Echinacea should be administered with caution concomitantly with immunosupressant drugs.[37] 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 CY3A4 inhibition are nat systematically available the constituents that are responsible for CYP3A4 induction may rapidly be absorbed leading to lack of intestinal CY3A4 induction ,hepatic CYP3A4 induction may occure 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 testosteron by CYP3A4 nicotinamide adenine dinucleotide phosphate (NADPH) dependent reaction .[41]

8. DISCUISSION :

Echinacea purpurea has worldwide reputation for its immunomodulatory and antiinflammatory properties capable of modulating of various immune sysytem pathways . T here are different classes of secondary metabolites of the plant showing immunostimulatory activity such asalkamides ,caffeic acid derivatives,polysaccharides and glycoproteins. [42] The polar extracts of the plant usually contain more polar metabolites including polysachharides and glycoproteins. [43] Another compounds 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 seprate 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 accute toxicologically events are lacking .

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

9.USES :

9.1.Anti-infflamatory 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 carrageenal induced paw edema similar to indomethacin .[48] This effect may be atributed 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 detemined 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 sialogogue 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 dependant manner .This result represent starting point to establish vaible scientific evidence on the possible rolo 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. Pupurea extract the result reflectedthe deterimental 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 aqeous extract of E.Purpurea showed resistance to influenza A2 , herbs and vesicular stomatities infection for 24 hrs whwn incubeted on mouce fibro glass and also against Herpes simplex virus and influenza virus a high weight molecular fraction containing polysaccharide and glycoprotiens responsible for the activity .[55]

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