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# Antimicrobial activity of some medicinal plants endemic to North America /

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# ANTIMICROBIAL ACTIVITY OF SOME MEDICINAL PLANTS ENDEMIC TO NORTH AMERICA

A Thesis Presented

by

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ABSTRACT

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Essential oils extracted from fresh and dried roots and herb of *Echinacea purpurea* (L.) Moench, *E. pallida* Nutt. and *E. angustifolia* DC, and fresh herb of *Chenopodium ambrosioides* L., were tested for antimicrobial activity against gram-positive *Staphylococcus aureus* (ATCC 25923) and *Enterococcus faecalis* (ATCC 19433), gram-negative *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853), and the yeast *Candida albicans* (ATCC 14053). Antimicrobial screening was done on essential oils, extracted by a simultaneous steam distillation-solvent extraction procedure, using a qualitative agar diffusion test. Essential oil of *Chenopodium ambrosioides* was active against *Candida albicans* only. Essential oils of *Echinacea* species were active against all microorganisms tested. *Candida albicans* was the microorganism, most inhibited by the essential oils. Gram-negative bacteria were less sensitive to the action of essential oils than gram-positive. Overall, essential oils extracted from dried roots and herb of *Echinacea* species had more antimicrobial activity

than oils extracted from fresh roots and herb. Gram-positive *Enterococcus faecalis* was sensitive only to oils extracted from fresh roots and herb of *Echinacea* species.

Keywords: essential oils, *Echinacea, Chenopodium ambrosioides.*

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# CHAPTER I

# INTRODUCTION

Biologically active, secondary metabolites from higher plants represent an important source of drugs used in a natural unmodified form or as templates for the synthesis of analogues with improved pharmacological activity or reduced toxicity (Farnsworth, 1984; Kinghorn, 1994; Borris, 1996; Soejarto, 1996). With the emergence of microbes resistance to antibiotics during the last decade, the possibility of plant containing novel antimicrobial compounds for pharmaceutical use has emerged (Berkowitz,1995; Jacoby, 1984). In this respect, the tremendous chemical diversity of higher plants will need to be evaluated for antimicrobial activity.

Various screening methods for evaluation of antimicrobial properties of plant extracts have been reported (Mitscher et al., 1972; Brantner and Grein, 1994). By using specific indicator organisms predictive of possible utility against human bacterial diseases, Mitscher and Raghav Rao (1984) were able to detect reproducible antimicrobial activity in one in five extracts from wide variety of plant genera. According to Tschesche (1971), after investigation of 139 different plant species, 83 percent showed antibiotic activity with 29 percent active against bacteria, 13 percent active against fungi, and 40 percent active against both groups. Generally, flowers were the most active, with leaves, roots, and branches having less activity.

In many plants producing biologically active secondary metabolites, the essential or volatile oil fraction exhibited the most antimicrobial activity (Deans, 1991). Essential oils are volatile lipophylic substances with a characteristic aroma, composed mostly of

terpenes and phenylpropenes (Waterman, 1993). Essential oils play an ecological role in a plant and generally store isolated from normal physiological processes of the producing plant in extracellular spaces such as glands or ducts (Deans and Waterman, 1993).

The antimicrobial, antifungal and insecticidal properties of essential oils have been intensively investigated (Carta et al. 1996; Lis-Balchin et al. 1996; Deans and Ritchie, 1987; Safiyev et al.,1998; Duke, 1990; Mookherjee et al. 1993). Significant biological activity against wide range of bacteria, fungus, molds and yeast, as well as antioxidant and antiviral activities showed by essential oils, suggested their utilization in aromatherapy, pharmaceutical, cosmetic, and agricultural industries (Svoboda and Deans,1995; Dorman et al. 1995; Bishop, 1995; Deans and Waterman, 1993).

Various essential oils from aromatic plants are presently used as sedatives, expectorants, antiseptics, and disinfectants, in addition to use as flavoring agents in food industry and perfumery. Although the medical applications of essential oils are presently limited, essential oil producing medicinal plants are included in pharmacopoeias of many countries (Sticher,1977).

The objective of the present study was to investigate antimicrobial activity proposed for essential oils of *Echinacea* species and *Chenopodium ambrosioides* against human pathogenic bacteria.

# CHAPTER II

# LITERATURE REVIEW

Plants are considered to be medicinal if pharmacological activity with possible therapeutic use exists. An effective method for the discovery of new drugs has been the screening of selected plant extracts, based on ethnomedical information or traditional medicinal uses, with subsequent identification and isolation of the active compounds (Duke, 1990; Cavé, 1985). In fact, 74 percent of all plant-derived drugs in clinical use worldwide have been discovered through investigations of ethnomedical uses (Soejarto, 1996).

Although ethnobotanical research is widely recognized and has expanded greatly in recent years, only a few screenings of North American medicinal plants have ever been undertaken, leaving the medicinal properties of the vast majority of North American plants unknown (Foster, 1991; Tyler, 1996; McCutcheon et al, 1992). North American flora does contain a large number of medicinal plants used by the aboriginal people. Indeed, American Indian medicine was in use for thousands of years (Hutchens, 1991). Native Americans used almost 3, 000 of 31, 566 vascular species for the medicinal purposes. Plant families *Asteraceae, Rosaceae, Apiaceae, Ranunculaceae* and *Lamiaceae* were intensively utilized (Moerman, 1998).

According to Gilmore (1913), Native Americans used *Echinacea* (purple coneflower) for more purposes than any other medicinal plant, applying juice from fresh roots as an antidote for snakebites and other poisonous conditions, and for headaches, toothaches, sore throats, septic conditions, inflammations, eczema, ulcers and colds

(Vogel, 1970; Kindscher, 1989). In the late 19th and early 20th century a tincture of *Echinacea angustifolia* was the most widely used plant drug in the United States. The drug was used internally for the prophylactic and treatment of mild to moderately severe colds, in tumorous and syphilitic conditions, influenza, and septic conditions, and externally for the treatment of hard to heal wounds and skin inflammations (Lloyd, 1917). Interest in *Echinacea* as a medicinal plant has recently increased, supported by the scientific justification of biological activity.

*Echinacea (Asteracea)* contains nine species native to the United States and south central Canada and of these, three species are of medical importance (Fernald,1950; Foster, 1991) (Table 1). *Echinacea* species are also widely distributed as garden ornamentals with many cultivars developed for horticultural purposes (Foster, 1997).

In the past decades, numerous chemical and pharmacological studies have been done on *Echinacea* species. The protective effects of caffeoyl derivatives of *Echinacea* species (echinacoside, chlorogenic acid, chicoric acid, cynarine, and caffeic acid) on the free radical-induced degradation of Type III collagen, indicate potential topical use of *Echinacea* extracts species for the prevention and treatment of photodamage to the skin (Facino et al, 1995). The phenolic compound echinacoside isolated from *E. angustifolia* roots has been reported to have wound healing activity (Stoll et al,1950). A mixture of complex polysaccharides, named Echinacin B and isolated from *E. angustifolia* and *E. purpurea,* also appears to promote wound healing in experimental animals. Wound healing activity is attributed to the inhibition of hyaluronudase enzyme by the formation of a polysaccharide complex with hyaluronic acid that stimulates the growth of fibroblasts, the connective tissue forming cells (Moring, 1983). Topical applications of

isolated crude polysaccharide mixture demonstrated "cortisone like activity," and displayed anti-inflammatory activity in a rat paw oedema model after (Bauer and Wagner, 1991). Other experiments utilized extracts of roots and herbs of *Echinacea purpurea* have suggested possible effects of the extracts on the environmental matrix of the cells active in the healing process, e.g., leukocytes, macrophages, and T-lymphocytes (Zoutewelle and van Wijk, 1990).

In an infection stress test, *E. purpurea* polysaccharides protected mice infected with *Candida albicans*, and enhanced the survival rate of lethal *Candida* infections to 100 percent (R. Bauer, 1998). Caffeic acid derivative echinacoside from the roots of *E. angustifolia* have demonstrated mild antibacterial activity against *Staphylococcus aureus* and *Streptococcus pyogenes* (Stoll et al, 1950).

Pentane soluble essential oil extracted from the roots of *E. angustifolia* and pentane extract of *E. pallida* roots inhibited the growth of Walker carcinosarcoma 256 and P-388 lymphocyte leukemia in mice (Bauer and Wagner, 1991). The active constituent with oncolytic properties has been isolated and identified as (Z)-1,8-pentadecadiene, the first diene olefine reported to possess *in vivo* antitumor activity (Voaden and Jacobson, 1972). Treatment of leucopenia patients undergoing radiotherapy with a phytopreparation of *Echinacea purpurea* and *Baptisia tinctoria* extracts significantly increased the number of leukocytes (Wagner, 1995).

*Echinacea* species are most known for immunostimulatory activities demonstrated in many *in vitro* experiments, including animal studies and clinical trials. Investigation on water and alcoholic extracts of *Echinacea* species isolated active constituents (including lipophilic alkymides and water soluble polysaccharides and

cichoric acid). The immunostimulatory activity of *Echinacea* species is attributed to non-specific stimulation of the immune system, based on stimulation of phagocytosis and activation of lymphocytes, causing increased elimination of pathogenic bacteria, viruses, and toxins produced in the bloodstream (Houghton, 1994).

Significant reduction in viral and bacterial infection susceptibility and inflammatory laryngitis was reported in 160 patients suffering from influenza after the treatment with *E. pallida* root extract (Wagner, 1995). In a clinical trial with 32 patients with a common cold those treated with *E. purpurea* root extract preparation recovered faster than those in a control group (Scaglione and Lund, 1995). In an *in vivo* experiment with oral administration of ethanolic extracts of *Echinacea purpurea*, *E. angustifolia* and *E. pallida* significant stimulation of phagocytosis in mice was reported (Bauer et al, 1988). The lipophilic fractions of the extracts appeared to be more active than the polar fractions (Bauer et al, 1988).

The polysaccharide arabinogalactan and arabinigalactan-containing glycoproteins isolated from roots of *Echinacea* species have been proven to be responsible for the immunostimulating activity. The isolated polysaccharides from all three species exhibited a high degree of similarity in immunomodulating and antiviral effects, with the *E. purpurea* extracts exerting the strongest potency (Willigmann et al, 1993; Bodinet et al, 1993). Applications of purified polysaccharides from *E. purpurea* to mice resulted in significant protection against the lethal infections with the macrophage-dependent *Listeria monocytogenes* and granulocyte-dependent *Candida albicans* (Roesler et al, 1991).

According to Stimpel et al (1984), macrophages activated by polysaccharide fractions of *E. purpurea* develop distinctive extracellular cytotoxicity against tumor cells. As a result of activation of the macrophages tumor necrosis factor and interferon were produced, suggesting therapeutic implications in the defense against tumors and infections (Luettig et al, 1989). In other study, purified glycoprotein-containing fractions showed indirect antiviral activity against vesicular stomatitis virus via an interferon induction, and direct antiviral effect against herpes simplex virus, causing a 100 percent plaque-reduction (Bodinet and Beuscher, 1991).

The study utilizing different models of stomach ulcers in rats revealed strong anti-ulcerous effect of the tinctures from fresh flowers and roots of *E. purpurea*, confirmed by clinical studies, and tinctures were recommended as effective remedy for ulcer treatment (Voitenko et al, 1995).

Clinical studies, involving 42 patients with proliferative arthritis, revealed significant reduction of clinical symptoms of rheumatoid joint inflammation after the treatment with non-steroid antiplogistics in combination with tincture of *E. purpurea*. Immuno-modulating activity of *E. purpurea* resulted in the increase of both lymphocyte content and the number of immunoglobulin of A type. The preparation was recommended for complex treatment of proliferative arthritis (Babunina et al, 1994; Hryzhak et al,1994).

Other medicinal plant used as a subject in this investigation, *Chenopodium ambrosioides* L. (*Chenopodiaceae*) is a native of tropical America but has widely established as a weed in gardens, roadsides and waste places in almost all parts of the United States. Commonly known as American wormseed, Mexican tea or epazote,

*Chenopodium ambrosioides* is annual erect plant growing 2 to 4 ft. high with yellowish-green flowers in spikes, leaves with tiny yellow glands, and dark brown shiny seeds (Cronquist and Gleason, 1991). The seeds ripen and are collected in the autumn. The whole plant and the essential oil extracted from leaves and seeds are distinguished by rank odor, and presently used as fragrance components in creams, lotions, perfumes and soaps. Traditionally, the plant was used in cooking as flavoring agent (Duke, 1985).

Folk uses by native Americans include applying the plant for fevers, headaches, rheumatism, swellings, toothaches, stomachaches, bronchitis, and as a tonic. The plant was used by rural population in Brazil in the treatment of skin ulceration due to *Leishmania (Viannia) braziliensis* (Franca et al, 1996).

*Chenopodium ambrosioides* is well known vermifuge. Tea from leaves and essential oil distilled from flowering and fruiting plant were widely used to expel roundworms, hookworms and intestinal ameba. The plant was considered as the most efficient and safe vermifuge, especially for children. The essential oil was officially included as an anthelmintic in the U. S. Pharmacopoeia before it was replaced by synthetic drugs as a result of difficulties in standardization of the active principle of the drug, and toxicity of the oil in high doses (Moerman, 1998; Kay, 1996; Vogel, 1970). Currently, the oil of *Chenopodium ambrosioides* is mainly used in veterinary practice. The plant is largely cultivated and oil is distilled in Maryland (Trease and Evans, 1983).

Recent studies revealed strong toxic effect of *Chenopodium ambrosioides* against *Rhizoctonia solani*, a plant pathogen responsible for severe damping-off diseases. Volatile oil from leaves of *Chenopodium* exhibited absolute inhibition of the mycelial growth of the fungus at 1000 ppm, and at 250 ppm in combination with other volatile oils

of *Lippia alba* and *Ocimum canum.* At the same time, the oil of *Chenopodium* did not exhibit any adverse effect on seed germination and seedling growth of the experimental host plant *Phaseolus aureus*. Leaves of *Chenopodium* incorporated with soil were found to control damping-off in crop seedlings by 70 percent. Considering antifungal potency and lack of phytotoxicity, essential oil of *Chenopodium ambrosioides* has been observed to be superior to several commonly used synthetic fungicides (Dubey and Kishore, 1987; Kishore et al, 1989). In other study, the plant exhibited allelopathic effects actually inhibiting seed germination and seedling growth of some weeds (Datta and Ghosh, 1987).

The essential oil of *Chenopodium ambrosioides* was reported to have absolute antifungal activity against nine human pathogenic dermatophytes at a concentration of 50 ppm (Kishore et al, 1996). Moreover, the oil retained the antidermatophytic activity for at least 360 days.

Crude ethanol extract from seeds of *Chenopodium ambrosioides* was observed to be active against stored grain pests *Sitophilis granarius* and *Tribolium castaneum* with 11 of the most active principles of the extract isolated and identified (Kandil et al, 1989). Other studies have confirmed repellent activity of *Chenopodium* ambrosioides oil against stored product insects (Su, 1991; Malik and Nagvi, 1984). Aqueous leaf extract of *Chenopodium ambrosioides* had prevented infection of tobacco mosaic and sunnhemp rosette viruses by indirectly increasing the resistance of the host plants, promoting the formation of certain virus interfering substances in the host (Verma and Baranwal, 1983).

Ascaridole, an asymmetric monoterpene endoperoxide with anthelmintic properties, is the main constituent (60-80 %) of the essential oil of *Chenopodium ambrosioides*. Naturally occurring endoperoxides from other plants are known for

antimalarial activity (Pollack et al, 1990), and in a study undertaken to determine antimalarial activity ascaridole was observed to be a potent inhibitor of plasmodial growth at low concentrations against *Plasmodium falciparum* (Pollack et al, 1990).

# CHAPTER III

# MATERIALS AND METHODS

## Plant material

*Echinacea* species (*Echinacea purpurea* (L.) Moench, *E. pallida* Nutt., and *E. angustifolia* DC) and *Chenopodium ambrosioides* L. plants were used in this study. Dried roots and herb of *Echinacea purpurea*, *E. pallida* and *E. angustifolia* were obtained from Trout Lake Farm (Trout Lake, WA). Additional *Echinacea* plants and *Chenopodium ambrosioides* were grown from seed (Richters, Ontario, Canada) in a greenhouse at the Department Plant and Soil Sciences, University of Massachusetts at Amherst, MA (42.2 ° North latitude) with full sunlight with a day temperature range of 23.0 - 35.0 °C, depending on outside temperatures, and a minimum night temperature of 19 °C. The seeds of the plants grown in the greenhouse were sown in a commercial potting mixture, ProMix BX (peat moss:vermiculite:perlite, 60:20:20 parts by volume, pH 6.0), contained in plastic pots (4.0 cm in diameter). At the seedling stage, fresh plants were transferred to larger plastic pots (22.5 cm in diameter). To maintain vigorous growth, plants were fertilized with 100 ml solution of Peat-Lite Special® with concentration of nitrogen at 350 ppm. Plants were harvested at flowering and fruiting stage (15 weeks after sowing for *Chenopodium ambrosioides* plants and 36 - 40 weeks after sowing for *Echinacea* plants) and the roots and tops separated from each other. The essential oil was immediately extracted from the fresh tissues, including stems, leaves, flowers, and seeds for *Chenopodium ambrosioides,* and roots, stems, leaves, and flowers for *Echinacea* plants (Fig. 1-7).

## Extraction

Extraction of essential oils was done by simultaneous steam distillation-solvent extraction (Likens and Nickerson, 1964, Koedam et al, 1979; Godefroot et al, 1981) (Fig. 8). Plant material (50 g) was transferred to a 500 ml distillation flask containing 250 ml of distilled water. The solvent n-pentane (25 ml) was added to 50 ml round-bottomed, receiving flask. Before starting the extraction, 2.5 ml of distilled water and 2 ml of n-pentane were introduced into the return arm of the unit. The reflux of pentane and plant material was started by heating the flasks at 50 °C and 100 °C, respectively, for smooth boiling in the sample and the solvent flasks. The vapors were captured in condensers with circulating ice-cold water above the unit. Condensed distillate and solvent dripped to U-tube of the return arm with separation of the two phases and return to respective flasks, resulting in all volatile material being collected in the solvent containing flask. At the end of the distillation procedure (one hour for *Echinacea* spp. and 30 minutes for *Chenopodium ambrosioides*), the n-pentane was removed from the distillate by rotary vacuum evaporation at 40 °C and under a nitrogen atmosphere. Essential oil samples were maintained under 5 °C until tested for antimicrobial activity.

## Test microorganisms

Gram-positive *Staphylococcus aureus* (ATCC 25923) and *Enterococcus faecalis* (ATCC 19433), Gram-negative *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853), and the yeast *Candida albicans* (ATCC 14053) were used as test organisms. Cultures of each test organism were maintained in tryptic soy broth (for bacteria) and potato dextrose broth (for yeast).

## Experimental

Antimicrobial activity of essential oils was evaluated using an agar diffusion test (Barry, 1976; Janssen et al, 1986). To prepare for the diffusion test, an inoculating loop full of each bacterium and the yeast culture was used to inoculate individual tube slants of tryptic soy agar (for bacteria) and potato dextrose agar (for the yeast) for overnight incubation at 37 °C. A suspension of each bacteria and yeast from the 24 h slant was subsequently prepared in sterile saline solution to obtain a turbidity equal to a McFarland standard 0.5 ($10^8$ CFU/ml) prior inoculation.

The prepared suspension of each microbe was inoculated by streaking on the surface of Mueller-Hinton agar contained in Petri plates (9 cm in diameter). A sterile filter paper disc (BBL® 0.6 cm in diameter) was placed on the agar surface. The essential oil, (5 µl of selected undiluted oil), was pipetted on the paper disc on the agar surface. An antibiotic containing disc (Vancomycin, Polymixin B, or Nystatin, depending on the microorganism) served as a positive control on the same plate, and a third disc impregnated with 5 µl of sterile distilled water (to serve as a negative control) was placed on the same plate. The three discs on each plate were equidistant from each other. After placement of the discs, the plates were inverted and incubated overnight at 37 °C. Antimicrobial activity was determined by measuring the diameter of the clear zone around each disc. All assays were done in triplicate.

## Statistical analysis

Data were analyzed using analysis of variance with Duncan's New Multiple-Range test used for separation of means.

# CHAPTER IV

# RESULTS

## Essential oil

The essential oil obtained from fresh herb of *Chenopodium ambrosioides* (2.6 % fresh weight) was colorless with a strong camphoraceous aroma (Table 2). The essential oils obtained from fresh herb and roots of *Echinacea* species (average of 0.05 %) were a light yellow color with a pleasant aroma. Fresh *Echinacea purpurea* herb contained the highest concentration of essential oil (0.2 % fresh weight). Fresh roots of *Echinacea pallida* contained a higher concentration of essential oil than roots from the other *Echinacea* species (0.08% fresh weight). Dried *Echinacea purpurea* herb had an essential oil concentration of 0.1 percent, a level lower than that of fresh herb. Dried roots of *Echinacea pallida* also contained a lower concentration of essential oil (0.07 %) than fresh roots of this species. Tissue from *Echinacea angustifolia* roots and herb had the lowest essential oil concentrations among the tested *Echinacea* species.

## Antimicrobial activity

The essential oil from fresh herb of *Chenopodium ambrosioides* demonstrated antimicrobial activity only against yeast (Table 3), but in comparison with the antibiotic nystatin was relatively ineffective in preventing growth of the yeast organism. In contrast, the essential oil from fresh and dried tissues of *Echinacea* species demonstrated significant antimicrobial activity against all microorganisms tested.

The essential oil from dried roots of *Echinacea purpurea* and *E. angustifolia* significantly inhibited the growth of gram-negative *E. coli,* superior to that exhibited by the antibiotic Polymixin B. Essential oils from dried roots of *Echinacea purpurea* and *E.*

*angustifolia* also inhibited the growth of the gram-negative *P. aeruginosa*, but the growth inhibition was less than that exhibited by the antibiotic Polymixin B. In contrast, the essential oils extracted from dried herb of the *Echinacea* species were more effective against *P. aeruginosa* than against *E. coli*. The essential oils from fresh roots tissue of *Echinacea* species demonstrated no inhibition of gram-negative bacteria. The essential oil from fresh herb of *Echinacea purpurea* inhibited the growth of *P. aeruginosa* only.

Essential oils from dried roots of *Echinacea* species were also active against the gram-positive *S. aureus*. The essential oil from dried roots of *Echinacea purpurea* inhibited the growth of *S. aureus* more than the essential oil from dried roots and herb of *E. pallida* and *E. angustifolia*. Essential oils extracted from fresh roots of *Echinacea* were less active against *S. aureus* than essential oils from dried roots. Only essential oil from fresh root of *Echinacea pallida* demonstrated antimicrobial activity against gram-positive *Enterococcus faecalis.*

The essential oils from dried herb of *Echinacea* species also inhibited the growth of *S. aureus*. The essential oil from dried herb of *Echinacea pallida* was more active than essential oils of dried herb of *E. pallida* and *E. angustifolia*. The essential oils from fresh herb of *Echinacea* species demonstrated less activity against *S. aureus*, but inhibited the growth of *Enterococcus faecalis.*

The essential oils extracted from *Echinacea* species had antimicrobial activity against the yeast *Candida albicans* with the level of inhibition superior to that of the antibiotic nystatin. Essential oils from dried roots of *Echinacea angustifolia* and *E. purpurea* exhibited the highest activity. In contrast, the essential oil from dried herb of *Echinacea pallida* inhibited the growth of *Candida albicans* more than essential oils from

dried herbs of *E. angustifolia* and *E. purpurea.* The essential oil from fresh herb of *E. angustifolia* inhibited the yeast organism more than essential oils from fresh herb of *E. pallida* and *E. purpurea.*

Overall, essential oils extracted from dried roots and herb tissue of *Echinacea* species had higher antimicrobial activity than essential oils extracted from fresh roots and herb tissue. In contrast, only essential oils extracted from fresh roots and herb tissue of *Echinacea* species inhibited the growth of the gram-positive bacterium *Enterococcus faecalis*.

# CHAPTER V

# DISSCUSION

## Essential oil

*Chenopodium ambrosioides* was previously reported to contain 0.4-1 percent of essential oil obtained from fresh herb, and 1-4 percent of essential oil obtained from seeds (Trease and Evans, 1983). According to Sagrero-Nieves and Bartley (1995), fresh leaves of *Chenopodium ambrosioides* yielded 1.2 percent of essential oil. In our study, fresh herb and seeds of *Chenopodium ambrosioides* were used for distillation of an essential oil, and the results obtained (2.6 percent of essential oil) confirm previous reports.

Analysis of essential oil content of *Echinacea* species by Bauer and Wagner (1991) showed that *Echinacea* species contain various amounts of the essential oil, with less than 0.1 percent of essential oil reported for *E. angustifolia*, and 0.01-0.6 percent of essential oil reported for *E. pallida* and *E. purpurea.* In our study, the least amount of an essential oil was also obtained from fresh and dried tissue of *Echinacea angustifolia,* and the highest amount of an essential oil was obtained from fresh herb (including flowers) of *E. purpurea* and from fresh roots of *E. pallida*. Minimal differences in essential oil yield of dried and fresh tissues observed in this study, where dried tissues of *Echinacea* species yielded same or higher amount of essential oil than fresh tissues, are probably due to high content of moisture in fresh tissues, contributing to the overall weight.

## Antimicrobial activity

Antifungal activity of *Chenopodium ambrosioides* essential oil against the pathogenic yeast *Candida albicans* supports the broad antifungal activity of the plant previously reported against plant and human pathogenic fungi (N. Kishore et al, 1989; N. Kishore et al, 1996). *Candida albicans,* as a dimorphic fungus, is able to grow in the yeast and mycelium forms, depending on environmental conditions. As human and animal pathogen, *Candida albicans* often occurs in systemic infections in the mycelial form, which resist phagocytosis (Saltarelli, 1989). Total inhibition of the growth of other yeast species *Candida utilis*, *C. tropicalis* and *C. lipolytica* by essential oils of *Origanum* species was reported previously (Charai, 1996). According to Adams et al (1996), significant inhibition of fungal mycelia and deformation of fungal cell wall composition of the common food contaminating fungus have been observed after treatment with some essential oil components.

Antifungal action of the essential oil of *Chenopodium ambrosioides* has been attributed to ascaridol, the unsaturated monoterpene peroxide, which constitutes about 70 to 90 percent of the essential oil (Duke, 1985; Williamson and Evans, 1988). In contrast, limonene and trans-pinocarveol were identified as major compounds of the oil of *Chenopodium ambrosioides* growing in Mexico, comprising respectively 32.5 and 26.7 percent of the total oil (Sagrero-Nieves and J. P. Bartley, 1995). In my opinion, lack of detectable amounts of ascaridol reported by the authors above is due to the long (3 hours) steam distillation procedure used for the extraction of the essential oil. Ascaridol was reported to gradually decompose while boiling with water, and rapid distillation procedures have been suggested (Trease and Evans, 1983).

Lack of antibacterial activity of the oil of *Chenopodium ambrosioides* against gram-positive and gram-negative microorganisms in this study is probably due to lack of phenolic and alcoholic compounds in the oil, compounds that are reported to be responsible for the antibacterial activity of essential oils (Knobloch et al, 1986). According to Kishore *et al* (1996), no detectable phenols were observed in essential oil of *Chenopodium ambrosioides*. Additional studies will be needed to evaluate the chemical composition of the essential oil of *Chenopodium ambrosioides* in reference to the biological activity.

Significant antifungal activity of *Echinacea* essential oils against *Candida albicans* exerted in this study suggest conducting more detailed investigations in the future considering importance of the microorganism as human opportunistic pathogen. Clinical studies have previously demonstrated the efficacy of the extracts of *Echinacea* species against recurrence of vaginal candidiasis (Houghton, 1994). The results of this study also suggest additional investigations of the potential antifungal activity of *Echinacea* essential oils against other human and plant pathogenic fungi.

In our study, gram-negative bacteria used as the test microorganisms were more resistant to the essential oils of *Echinacea* than gram-positive. Different sensitivity of gram-negative and gram-positive bacteria may be due to differences in cell wall structure with an additional outer lipid bilayer membrane around gram-negative bacteria cell wall, although being lipid soluble essential oil compounds would be expected to easily penetrate through the cell wall. According to Knobloch *et al* (1986), the site of antimicrobial action of essential oil compounds occurs within the bacterial cytoplasm respiratory system, where functional groups of essential oil compounds can successfully

interfere with the process of energy metabolism. Based on our results, the growth of gram-positive *Enterococcus faecalis* was inhibited only by essential oils extracted from fresh tissues of *Echinacea* species. Structural specificity of *Enterococcus faecalis* as group D streptococcus (Sneath et al, 1986) probably makes the microorganism more sensitive to chemical constituents of essential oils extracted from fresh tissues.

The essential oils from aerial parts of *Echinacea* species are reported to contain borneol, bornyl acetate, polyacetylenes, germacrene D, caryophyllene, caryophyllene epoxide and palmitic acid (Bauer and Wagner, 1991). In addition, the achenes of *Echinacea* species are reported to contain α-pinene, β-pinene, myrcene, β-fernesene and limonene (Bauer and Wagner, 1991). Polyacetylenes in the essential oils isolated from *Echinacea purpurea* and *E. angustifolia* roots have previously demonstrated bacteriostatic activity against *Escherichia coli* and *Pseudomonas aeruginosa* (Bauer and Wagner, 1991). The antimicrobial properties of various essential oils constituents have been previously reported (Cruz et al, 1993; Deans, 1991). According to Chalchat and Garry (1997), certain patterns linking the composition and antimicrobial activity of the essential oils have been noted, where African essential oils, containing α-pinene, β-pinene, myrcene, limonene, germacrene D and caryophyllene as major constituents, display antimicrobial activity against six microbial strains, including gram-positive *Staphylococcus aureus*, gram-negative *Escherichia coli* and *Pseudomonas aeruginosa*, and *Candida albicans*. The present study suggests more detailed investigations are necessary to evaluate the active principles of essential oils of *Echinacea* species possessing antimicrobial properties.

Significant differences in antimicrobial activity between essential oils extracted from dried and fresh tissues of *Echinacea* species observed in this study are probably due to chemical decomposition of the essential oil compounds during drying procedure and long term storage of the plant material. According to Bauer and Wagner (1991), detailed investigations on essential oil compounds demonstrated that artifacts were produced by autoxidation of the native constituents in stored roots of *E. pallida*. No detectable polyacetylenes were observed in fragmented root material of *E. angustifolia* after long term storage. Germacrene alcohol, a component of essential oil from fresh aerial parts of *E. purpurea,* has not been detected in dried tissues.

In this study, any chemical decomposition of *Echinacea* essential oil constituents favored microbial inhibition properties, as oils extracted from dried plant tissues had higher antimicrobial activity than oils extracted from fresh plant tissues. Additional studies will be needed to evaluate qualitative differences in essential oil composition of dried and fresh tissues of *Echinacea* species.

Significant antimicrobial properties of essential oils isolated from *Echinacea* species in the present study support traditional uses of the plant by Native Americans in septic conditions, sore throats and skin inflammations, associated with bacterial infections.

Table 1. *Echinacea* species of medical importance.

| Specie | Botanical characteristics | Distribution |
|---|---|---|
| *Echinacea purpurea* (L.) Moench | Perennial, 2-5 feet high, stems rough-bristly, leaves rough, coarsely toothed, lowest leaves ovale, veiny, long-petioled, upper leaves oval-lanceolate, ray flowers rose to deep purple, pales with bright orange tips, fibrous roots. Flowers June-October | Dry open woods, prairies. Iowa, Illinois, Michigan, Ohio and Virginia, south to Oklahoma, Georgia and Louisiana. |
| *E. angustifolia* DC. | Perennial 5-20 inches high, stems rough-bristly, leaves lanceolate, entire, 3-nerved, ray flowers purple, very short, spreading, tap root. Flowers May-August | Prairies and barrens. Minnesota, Saskatchewan, south to Oklahoma, Texas. |
| *E. pallida* Nutt. | Perennial 5-40 inches high, stems with slender hairs, leaves lanceolate, ray flowers strongly drooping, curving toward the stem, pink to purple, white pollen, tap root. Flowers June-July | Prairies and barrens. Michigan to Nebraska, south to Alabama, Texas, Louisiana. |

Table 2. Essential oil yields.

| Plant | Plant part[1] | Essential oil yield (% of fresh or dried sample)[2] |
|---|---|---|
| *Echinacea pallida* | fresh herb[3] | 0.02 |
| | fresh root | 0.08 |
| | dried herb | 0.05 |
| | dried root | 0.07 |
| *E. purpurea* | fresh herb | 0.20 |
| | fresh root | 0.02 |
| | dried herb | 0.10 |
| | dried root | 0.02 |
| *E. angustifolia* | fresh herb | 0.01 |
| | fresh root | 0.01 |
| | dried herb | 0.05 |
| | dried root | 0.02 |
| *Chenopodium ambrosioides* | fresh herb | 2.60 |

[1] Fresh and dried material come from different sources and cannot be directly compared.
[2] Differences in concentration of essential oil in fresh material as compared with dried material probably result from differences in plant source; differences are consistent with other reports (Bauer and Wagner, 1991).
[3] Herb defines the aboveground part, including stems, leaves, and flowers.

Table 3. Antimicrobial activity of essential oils.

| Essential oil (5 µl) | Microorganisms | | | | |
|---|---|---|---|---|---|
| | *S. aureus* | *E. faecalis* | *E. coli* | *P. aeruginosa* | *C. albicans* |
| | Diameter of inhibition zone (mm)[2] | | | | |
| *Echinacea pallida* | | | | | |
| dried roots | 13.0[1] abc | -[3] | 8.5 c | - | 14.5 fg |
| fresh roots | 10.5 bc | 13.5 ab | - | - | 22.0 def |
| dried herb | 15.5 a | - | 7.5 c | 11.5 b | 41.5 b |
| fresh herb | 10.5 bc | 9.5 b | - | - | 24.5 cde |
| *E. purpurea* | | | | | |
| dried roots | 15.5 a | - | 25.5 a | 14.5 a | 45.0 ab |
| fresh roots | 9.5 c | - | - | - | 15.5 efg |
| dried herb | 14.5 ab | - | - | 8.5 c | 17.0 efg |
| fresh herb | 11.5 abc | 14.0 a | - | 8.5 c | 13.5 fg |
| *E. angustifolia* | | | | | |
| dried roots | 11.0 abc | - | 18.0 b | 14.5 a | 52.0 a |
| fresh root | - | - | - | - | 10.0 g |
| dried herb | 14.5 ab | - | - | 8.5 c | 31.0 c |
| fresh herb | 10.5 bc | 9.5 b | - | - | 29.5 cd |
| *C. ambrosioides* | | | | | |
| fresh herb | - | - | - | - | 21.5 def |
| Positive control | 20.0 | 20.0 | 15.0 | 15.0 | 25.0 |
| | Vancomycin 30 mcg | | Polymyxin B 300 Units | | Nystatin 100 Units |
| Negative control (distilled water) | - | - | - | - | - |

[1] Mean of 3 replications; means with the same letter within the column are not significantly different by Duncan's New Multiple Range Test ($p \leq 0.05$).
[2] Includes diameter of the paper disc (6 mm).
[3] No inhibition of microbial growth.

Figure 1. *Chenopodium ambrosioides* L.

Figure 2. *Echinacea pallida* Nutt.

Figure 3. *Echinacea purpurea* (L.) Moench

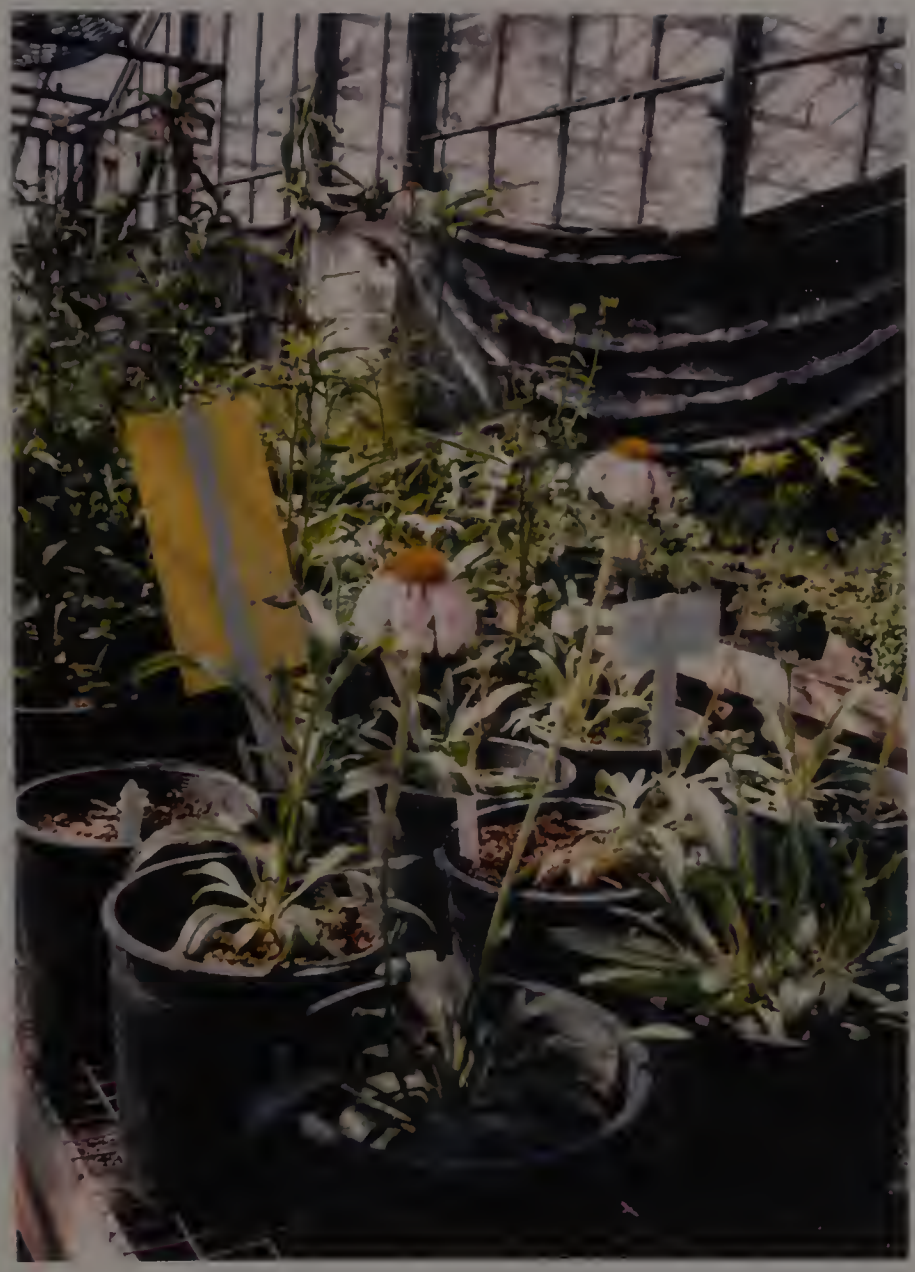

Figure 4. *Echinacea angustifolia* DC.


Figure 5. *Echinacea pallida* roots.


Figure 6. *Echinacea purpurea* roots.

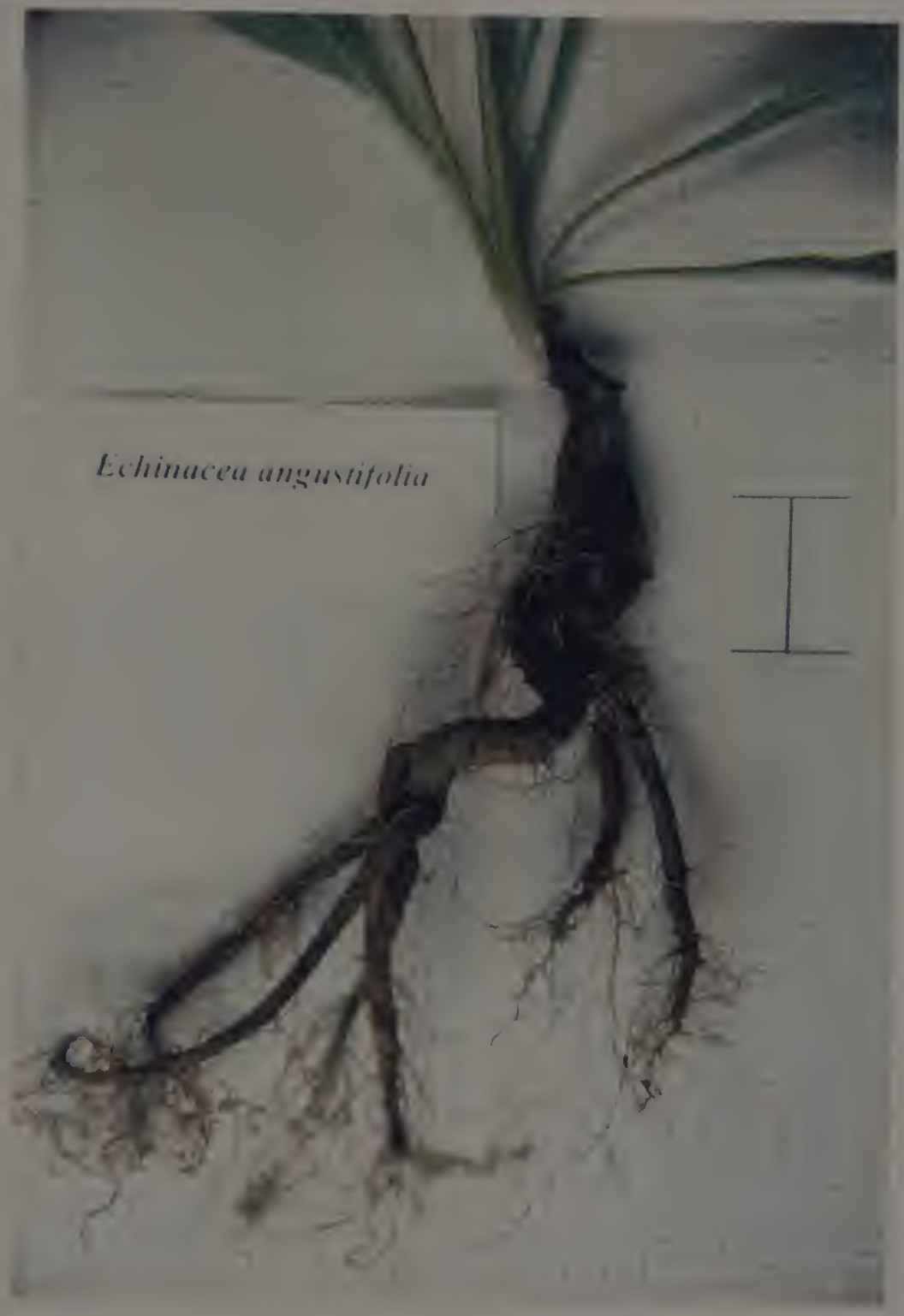


Figure 7. *Echinacea angustifolia* roots.

Figure 8. Simultaneous steam distillation-solvent extraction unit.

# BIBLIOGRAPHY


Adams, S., Kunz, B. And Weidenbörner, M. Mycelial deformations of Cladosporum herbarum due to the application of eugenol or carvacrol. *J. Essent. Oil Res.* 8:535-540, 1996.

Babunina, L. Y., Voitenko, H. M. and Bentsa, T. M.. Immunomodulating activity of nastoyka Echinacea purpurea in treatment of proliferate arthritis. Kiev: *Farmatsevtychnyi Zhurnal* 4:104-107, 1994.

Barry, A. The Antimicrobic Susceptibility Test: Principles and Practices. Philadelphia: Lea & Febiger, 1976.

Bauer, R. Echinacea: biological effects and active principles. In L. D. Lawson and R. Bauer, eds. *Phytomedicines of Europe: Chemistry and Biological activity.* Washington, DC: American Chemical Society, pp. 140-157, 1998.

Bauer, R. and Wagner, H. Echinacea species as potential immunostimulatory drugs. *Economic and Medicinal Plant Research* 5:253-321, 1991.

Bauer, V. R., Jurcic, K., Puhlmann, J., and Wagner, H. Immunologic in vivo and in vitro studies on Echinacea extracts. *Arzneimittelforschung* 38(2):276-281, 1988.

Berkowitz, F. E. Antibiotic resistance in bacteria. *Southern Medical Journal* 88(8):797-804, 1995.

Bishop, C. D. Antiviral activity of the essential oil of Melaleuca alternifolia (Maiden & Betche) Cheel (Tea Tree) against tobacco mosaic virus. *J. Essent. Oil Res.* 7:641-644, 1995.

Bodinet, C. Willigmann, I., and Beuscher, N. Host-resistance increasing activity of root extracts from Echinacea species. *Planta medica* 59(7):A672-A673, 1993.

Bodinet, C. and Beuscher, N. Antiviral and immunological activity of glycoproteins from Echinacea purpurea radix. *Planta medica* 57(2):A33-A43, 1991.

Borris, R. P. Natural products research: perspectives from a major pharmaceutical company. *J. of Ethnopharmacology* 51:29-38, 1996.

Brantner, A. and Grein, E. Antibacterial activity of plant extracts used externally in traditional medicine. *J. of Ethnopharmacology* 44:35-40, 1994.

Carta, C., Moretti, M., and Peana, A. Activity of the oil of Salvia officinalis L. against Botrytis cinerea. *J. Essent. Oil Res.* 8:399-404, 1996.

Cavé A. Methodology of research on medicinal plants. In D. Barton and W. D. Ollis, eds. *Advances in Medicinal Phytochemistry*. 47-56, 1985.

Chalchat, J.-C. and Garry, R.-Ph. Correlation between chemical composition and antimicrobial activity. VI. Activity of some African essential oils. *J. Essent. Oil Res*. 9:67-75, 1997.

Charai, M. and Mosaddak. M. Chemical composition and antimicrobial activities of two aromatic plants: Origanum majorana L. and O. Compactum Benth. *J. Essent. Oil Res*. 8:657-664, 1996.

Cronquist, A. and Gleason, H. Manual of Vascular Plants of Northeastern United States and Adjacent Canada. New York: New York Botanical Garden, 1991.

Cruz, T., Cabo, M.M., Castillo, M.J., and Jimenez, J. Chemical composition and antimicrobial activity of the essential oils of different samples of Thymus baeticus Boiss. *Phytotherapy Research* 7:92-94, 1993.

Datta, S. C. and Ghosh, K. N. Allelopathy in two species of Chenopodium - inhibition of germination and seedling growth of certain weeds. *Acta Societatis Botanicorum Poloniae* 56(2):257-270, 1987.

Deans, S. G. and Waterman, P. G. Biological activity of volatile oils. In R. K. M. Hay and P. G. Waterman, eds. *Volatile Oil Crops: their Biology, Biochemistry and Production*. England: Longman Scientific and Technical, pp. 97-111, 1993.

Deans, S. G. Evaluation of antimicrobial activity of essential (volatile) oils. In H. F. Linskens and J. F. Jackson, eds. *Essential Oils and Waxes*. Berlin: Springer-Verlag, pp. 310-320, 1991.

Deans, S. G. and Ritchie, G. Antibacterial properties of plant essential oils. *Intern J. Food Microbiol*. 5:165-180, 1987.

Dorman, H. J. D., Deans, S. G., and Noble, R. C. Evaluation in vitro of plant essential oils as natural antioxidants. *J. Essent. Oil Res*. 7:645-651, 1995.

Dubey, N. K. and N. Kishore. Fungitoxicity of some higher plants and synergistic activity of their essential oils. *Trop. Science*. 27:23-27, 1987.

Duke, S. O. Natural pesticides from plants. In J. Janick and J. E. Simon, eds. *Advances in New Crops*. Oregon: Timber Press, 511-517,1990.

Duke, J. Promising phytomedicinals. In J. Janick and J. E. Simon, eds. *Advances in New Crops*. Oregon: Timber Press, 491-498, 1990.

Duke, J. Handbook of Medicinal Herbs. London: CRC Press, 1985.

Facino, R. M. et al. Echinacoside and caffeoyl conjugates protect collagen from free radical-induced degradation: a potential use of Echinacea extracts in the prevention of skin photodamage. *Planta Medica* 61:510-514, 1995.

Farnsworth, N. R. The role of medicinal plants in drug development. In P. Krogsgaard-Larsen, S. Brögger Christensen, and H. Kofod, eds. *Natural products and drug development*. Copenhagen: Munksgaard, 17-30,1984.

Fernald, M. L. Gray's Manual of Botany. New York: American Book Company, 1950.

Foster, S. Herbal Renaissance. Salt Lake City: Gibbs Smith Publisher, 1997.

Foster, S. Echinacea. Nature's Immune Enhancer. Rochester: Healing Arts Press, 1991.

Franca, F., Lago, E. L., and Marsden, P. D. Plants used in the treatment of leishmanial ulcers due to Leishmania (Viannia) braziliensis in an endemic area of Bahia, Brazil. *Revista Da Sociedade Brasileira de Medicina Tropical* 29(3):229-232, 1996.

Gilmore, M. A study in the ethnobotany of the Omaha Indians. *Collect. Nebraska State Hist. Soc.* 17:314-357, 1913.

Godefroot, M., Sandra, P., and Verzele, M. New method for quantitative essential oil analysis. *J. of Chromatography* 203:325-335, 1981.

Houghton, P. Echinacea. *The Pharmaceutical Journal* 253:342-343, 1994.

Hryzhak, M. V. et al. The state of peroxide oxidation of lipids and antioxidising system in patients having proliferate arthritis when using complex treatment by non-steroid anti-inflammatory remedies in combination with nastoyka Echinacea. Kiev: *Farmatsevtychnyi Zhurnal* 3:76-78, 1994.

Hutchens, A. Indian Herbalogy of North America. Boston: Shambhala, 1991.

Janssen, A. M., Scheffer, J. J., and Baercheim Svendsen, A. Antimicrobial screening of essential oils - aspects of the agar overlay technique. In E.-J. Brunke, ed. *Progress in Essential Oils Research.* Berlin: Walter de Gruyter & Co., 401-419, 1986.

Kandil, M A., Abdallah, M. D., Farag, A.-A., and G. S. Peterson. Isolation and characterization of biologically active compounds from some plant extracts. *Pestic. Science* 25:337-342, 1989.

Kay, M. A. Healing with plants in the American and Mexican West. Tucson: University of Arizona Press, 132-134, 1996.

Kindscher, K. Ethnobotany of purple coneflower (Echinacea angustifolia, Asteracea) and other Echinacea species. *Economic Botany* 43(4):498-507, 1989.

Kinghorn, A. D. The discovery of drugs from higher plants. In Vincent P. Gullo, ed. *The discovery of natural products with therapeutic potential*. Boston: Butterworth-Heinemann, 81-108, 1994.

Kishore, N., Dixit, S. N., and Dubey, N. K. Fungitoxic studies with Chenopodium ambrosioides for control of damping off in Phaseolus aureus (Moong) caused by Rhizoctonia solani. *Trop. Science* 29:171-176, 1989.

Kishore, N., Chansouria, J. P. N., and Dubey, N. K. Antidermatophytic action of the essential oil of Chenopodium ambrosioides and an ointment prepared from it. *Phytotherapy Research* 10:453-455, 1996.

Knobloch, K., Weis, N., and Weigand, H. Mechanism of antimicrobial activity of essential oils. *Planta medica* 52:P114, 1986.

Knobloch, K., Weigand, H., Weis, N., Schwarm, H.-M., and Vigenschow, H. Action of terpenoids on energy metabolism. In E.-J. Brunke, ed. *Progress in Essential Oils Research*. Berlin: Walter de Gruyter & Co., 429-445, 1986.

Koedam, A., Scheffer, J. J. C., and Baerheim Svendsen, A. Comparison of isolation procedures for essential oils. 1. Dill (Anethum graveolens L.). *Chem. Mikrobiol. Technol. Lebensm.* 6:1-7, 1979.

Likens, S. T. and Nickerson, G. B. Detection of certain hop oil constituents in brewing products. *Amer. Soc. Brewing Chemists Proc.*5-13, 1964.

Lis-Balchin, M., Hart, S., Deans, S. G., and Eaglesham, E. Comparison of the pharmacological and antimicrobial action of commercial plant essential oils. *J. of Herbs, Spices and Medicinal Plants* 4(2):69-86, 1996.

Lloyd, J. U. A treatise on Echinacea angustifolia. Drug Treatise. Cincinnati: Lloyd Brothers Inc., 1917.

Luettig, B. Et al. Macrophage activation by the polysaccharide arabinogalactan isolated from plant cell cultures of Echinacea purpurea. *J. Nat. Cancer Inst.*81(9):669-675, 1989.

Malik, M.M. and Mujtabe Nagvi, S. H. Screening of some indigenous plants as repellents or antfeedants for stored grain insects. *J. of Stored Products Research* 20(1):41-44, 1984.

McCutcheon, A. R., Ellis, S.M., Hancock, R. E. W., and Towers, G.H.N. Antibiotic screening of medicinal plants of the British Columbian native peoples. *J. of Ethnopharmacology* 37:213-223, 1992.

Mitscher, L. A. and Raghav Rao, G. S. The search for new antimicrobial agents: unusual sources. In P. Krogsgaard-Larsen, S. Brögger Christensen, and H. Kofod, eds. *Natural Products and Drug Development*. Copenhagen: Munksgaard, 195-212, 1984.

Mitscher, L. et al. Antimicrobial agents from higher plants. 1. Introduction, rationale, and methodology. *Lloydia* 35(2):157-167, 1972.

Moerman, D. Native American Ethnobotany. Portland, Oregon: Timbler Press, 1998.

Mookherjee, B. D., Wilson, R., Schrankel, K. R., Katz, I., and Butler, J. F. Semio activity of flavor and fragrance molecules on various insect species. In R. Teranishi, R. Buttery, and H. Sugisawa, ed. *Bioactive Volatile Compounds from Plants*. Washington, DC: American Chemical Society, 35-48, 1993.

Moring, S. Echinacea: natural remedy for viral infections. The American Herb Association Newsletter 5-6, 1983.

Mundt, J. O. Enterococci. In P. H. A. Sneath, N. S. Mair, M. E. Sharpe, and J. G. Holt, ed. *Bergey's Manual of Systematic Bacteriology*. Baltimore: Williams & Wilkins, v.2, 1063-1064, 1986.

Pollack, Y., Segal, R., and Golenser, J. The effect of ascaridole on the vitro development of Plasmodium falciparum. *Parasitology Research* 76(7):570-572, 1990.

Roesler, J. et al. Application of purified polysaccharides from cell cultures of the plant Echinacea purpurea to mice mediates protection against systemic infections with Listeria monocytogenes and Candida albicans. *Int. J. Immunopharmacol* 13(1):27-37, 1991.

Safiyeva, S., Lisin, G., and Craker, L. E. Antimicrobial activity of some essential oils. *Acta Horticulturae*, 1998, in press.

Sagrero-Nieves, L. and Bartley, J. P. Volatile constituents from the leaves of Chenopodium ambrosioides L. *J. Essent. Oil Res*. 7:221-223, 1995.

Saltarelli, C. G. Candida albicans: The Pathogenic Fungus. New. York: Hemisphere Publishing Co., 1989.

Scaglione, F. and Lund, B. Efficacy in the treatment of the common cold of a preparation containing a Echinacea extract. *Inter. J. of Immunotherapy* 11(4):163-166, 1995.

Soejarto, D. Biodiversity prospecting and benefit sharing: perspectives from the field. *J. of Ethnopharmacology* 51:1-5, 1996.

Sticher, O. Plant mono-, di- and sesquiterpenoids with pharmacological or therapeutical activity. In H. Wagner and P. Wolf, ed. *New Natural Products and Plant Drugs with pharmacological, Biological or Therapeutical Activity*. Berlin: Springer-Verlag, 137-150, 1977.

Stimpel, M., Proksch, A., Wagner H., and Lohmann-Matthes, M. L. Macrophage activation and induction of macrophage cytotoxicity by purified polysaccharide fractions from the plant Echinacea purpurea. *Infect. Immun.* 46(3):845-849, 1984.

Stoll, A., Renz, J., and Brack, A. Isoliering und konstitution des echinacosids, eines glycosids aus den Wurzeln von Echinacea angustifolia D. C. *Helvetica Chimica Acta* 33:1877-1893, 1950.

Su, H. C. F. Toxicity and repellency of Chenopodium oil to four species of stored product insects. *J. of Entomological Science* 26(1):178-182, 1991.

Svoboda, K. P. and Deans, S. G. Biological activities of essential oils from selected aromatic plants. *Acta Horticulturae* 390:203-209, 1995.

Svoboda, G. H. Recent advances in the search for antitumor agents of plant origin. In H. Wagner and L. Hörhammer , ed. *Pharmacognozy and Phytochemistry*. Berlin: Springer-Verlag, 166-200, 1971.

Trease, G. E. and Evans, W. C. Pharmacognozy. London: Baillière Tindall, 1983.

Tschesche, R. Advances in the chemistry of antibiotic substances from higher plants. In H. Wagner and L. Hörhammer , eds. *Pharmacognozy and Phytochemistry*. Berlin: Springer-Verlag, 274-289, 1971.

Tubaro, A. et al. Anti-inflammatory activity of a polysaccharide fraction of Echinacea angustifolia. *J. Pharmacol.* 39(7):567-569, 1987.

Tyler, V. Pharmacognozy? What is that? You spell it now? *Economic Botany* 50(1):3-9, 1996.

Verma, H. N. And Barahwal, V. K. Antiviral activity and the physical properties of the leaf extract of Chenopodium ambrosioides L. *Proc. Indian Acad. Science (Plant Science)* 92(6):461-465, 1983.

Voaden, D. J. and Jacobson, M. Tumor inhibitors. 3. Identification and synthesis of an oncolytic hydrocarbon from American coneflower roots. *J. of Medicinal Chemistry* 15(6):619-623, 1972.

Vogel, V. J. American Indian Medicine. Norman: University of Oklahoma Press, 1970.

Voitenko, H. M., Varchenko, V. H., Lipkan, H. M., and Olinychenko, P. I. Influence of preparations made from roots and flowers of Echinacea purpurea over formation of experimental stomach dystrophy. Kiev: *Farmatsevtychnyi Zhurnal* 5:86-92, 1995.

Wagner, H. K. M. Immunostimulants and adaptogens from plants. In J. T. Arnason, R. Mata, and J. T. Romeo, ed. *Phytochemistry of Medicinal Plants*. New York: Plenum Press, 1-18, 1995.

Waterman, P. G. The chemistry of volatile oils. In R. K. M. Hay and P. G. Waterman, ed. *Volatile Oil Crops: their Biology, Biochemistry and Production*. England: Longman Scientific and Technical, 47-61, 1993.

Williamson, E. M. and Evans, F. J. Potter's New Cyclopaedia of Botanical Drugs and Preparations. Essex, England: Saffron Walden The C. W. Daniel Company Limited, 1988.

Willigmann, I., Egert, D., Bodinet, C., and Beuscher, N. Chemical and immunological properties of the immunomodulatory active compounds from the roots of different Echinacea species. *Planta Medica* 59(7):A671-A672, 1993.

Zoutewelle, G. and van Wijk, R. Effects of Echinacea purpurea extracts on fibroblast populated collagen lattice contraction. *Phytotherapy Research* 4(2):77-81, 1990.