RESEARCH PLAN PROPOSAL

PHYTOCHEMICAL STUDIES OF SELECTED TAXA OF ASTERACEAE AND THEIR BACTERICIDAL ACTIVITY

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INTRODUCTION

Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids and flavanoids which have been found *in vitro* to have antimicrobial properties. Laboratories all over the world have found literally thousands of phytochemicals which have inhibitory effects on all types of micro-organisms *in vitro* (Cown 1999). Phytochemistry is in the strict sense of the word the study of phytochemicals. These are chemicals derived from plants. Techniques commonly used in the field of phytochemistry are extraction, isolation and structural elucidation (MS, 1D and 2D NMR) of natural products, as well as various chromatography techniques (MPLC, HPLC, LC-MS). Phytochemistry is widely used in the field of indigenous medicine especially in the field of herbal medicine. For thousands of years, medicine depended exclusively on leaves, flowers and barks of plants. Only recently have synthetic drugs come into use and in

many instances, these are carbon copies of chemicals identified in plants (Conway, 1973). Hence phytochemical methods are important to screen and analyze bioactive components, not only for the quality control of crude drugs but also for the elucidation of their therapeutic mechanism.

The Asteraceae or Compositae (commonly referred to as the aster, daisy, or sunflower family), is an exceedingly large and widespread family of vascular plants. The group has more than 22,750 currently accepted species, spread across 1620 genera and 12 subfamilies. Along with the Orchidaceae this makes it one of the two largest flowering plant families in the world.

The largest composite genera are *Senecio* (1,000 species), *Vernonia* (1,000 species), *Centaurea* (700 species), *Cousinia* (600 species), *Helichrysum* (550 species) and *Artemisia* (550 species). Plants roots are usually taproots and sometimes fibrous. Stems are generally erect, but sometimes prostrate to ascending. Some species have underground stems in the form of caudices or rhizomes. The leaves and the stems very often contain secretory canals with resin or latex (particularly common among the Cichorioideae). The leaves can be alternate, opposite or whorled. They may be simple, but are often deeply lobed or otherwise incised often conduplicated or revolute. The margins can be entire or dentate. Asteraceae are especially common in open and dry environments. Many members of the Asteraceae are pollinated by insects, which explains their value in attracting beneficial insects, but anemophily is also present (e.g. *Ambrosia* and *Artemisia*). There are many apomictic species in the family. They produce iso/chlorogenic acid, sesquiterpene lactones, pentacyclic triterpene alcohols, various alkaloids, acetylenes (cyclic, aromatic, with vinyl end groups) and tannins, They have terpenoid essential oils which never contain Iridoids. In India members of some Asteraceae family and their therapeutic effects are:

Scientific Name	Common Name	Part of plant	Therapeutic effect
Arnica montana	Arnica	Flowers	relieve muscle pain
Arctium lappa	Burdock	Root	nourishes deep health
Eupatorium perfoliatum	Boneset	Herb	banishes flu
Calendula officinalis	Calendula	Flowers	salve wounds
Anthemis nobilis or *Matricaria chamomilla*	Chamomile	Flowers	soothes baby
Cichorium intybus	Chicory	Root	strengthens the liver
Tussilago farfara	Coltsfoot	Flowers	ease coughs
Taraxacum officinalis	Dandelion	Herb	improves liver function
Echinacea augustifolia	Echinacea	Root	counters bacterial infections
Inula helenium	Elecampane	Root	lung healer
Chrysanthemum parthenium	Feverfew	Leaves	prevents migraines
Grindelia robusta	Grindelia	Flower	opens breathing, stops itching
Senecio aureus	Liferoot	Flower	counters severe menstrual pain
Silybum marianum	Milk thistle	Seed	prevents liver distress
Artemisia vulgaris	Mug/cronewort	Herb	old woman's friend
Eupatorium purpurea	Queen of the meadow/gravel	Root	helps the kidneys
Tanacetum vulgare	Tansy	Flowers	repel insects
Achillea millefolium	Yarrow	Flowers	heal wounds, prevent colds
Valeriana officinalis	Valerian	Root	brings sleep
Artemisia absinthemum	Wormwood	Herb	prevents parasites
Lactucca species	Wild lettuce	Sap	eases severe pain

The present work involves phytochemical studies of three commonly occurring plants of Asteraceae family in Rajasthan.

1. ***Calendula officinalis***

Calendula, commonly known as pot marigold, is a genus of about 12–20 species of annual or perennial herbaceous plants in the daisy family of Asteraceae. It is native to the area from Macaronesia in the east through the Mediterranean region to Iran. The most commonly cultivated

and used member of the genus is the pot marigold (*C. officinalis*). The flowers of *C. officinalis* contain flavanol glycosides, triterpene oligoglycosides, oleanane-type triterpene glycosides, saponins, and a sesquiterpene glucoside.

Plant pharmacological studies have suggested that *Calendula* extracts have anti-viral, anti-genotoxic, and anti-inflammatory properties. *Calendula* in suspension or in tincture is used topically to treat acne, reducing inflammation, controlling bleeding, and soothing irritated tissue. There is limited evidence that calendula cream or ointment is effective in treating radiation dermatitis. *Calendula* has been used traditionally for abdominal cramps and constipation. An aqueous extract of *Calendula officinalis* obtained by a novel extraction method has demonstrated anti-tumor (cytotoxic) activity and immune-modulatory properties (lymphocyte activation) *in vitro*, as well as anti-tumor activity in mice.

2. ***Ageratum conyzoides***

A. conyzoides is an annual herbaceous plant with long history of traditional, medicinal and agricultural uses in several countries of the world. The plant has been known since ancient times for its curative properties and has been utilized for the treatment of various ailments, such as burns and wounds, infectious disease and arthritis fever. It ranges from South-eastern North America to Central America but the centre of origin is in Central America and the Caribbean. *A. conyzoides* is now found in several countries in tropical and sub-tropical regions of the world. There is high variability in the secondary metabolites of *A. conyzoides* which includes flavanoids, alkaloids, cumarins, essential oils and tannins. Many of these are biologically active.

Aqueous extracts of leaves or whole plants have been used to treat colic, colds and fevers, diarrohea, rheumatism, spasms or as a tonic.

3. ***Verbesina encelioides***

Commonly known as 'golden crown beard', *V. encelioides*, is a weed introduced from the Eastern United States. It shows aggressive and dominant growth abilities. This plant can tolerate wide range of climatic conditions including drought and high temperature.

It is known that the weeds are important source of medicines for indigenous people. Various primary and secondary metabolites like terpenoids, sesquiterpenes, flavonol glycosides and triterpenoids have been found in various parts of the plant body.

Extracts of these species exhibit anti-microbial, anti-viral, anti-tumor, hypoglycaemic and anti-implantation efficacies.

OBJECTIVES

Considering the above facts, this research work entitled, "Phytochemical studies of selected taxa of Asteraceae and their bactericidal activity" has been taken up with the following objectives:

1. To collect healthy plants and confirm them from voucher specimen in the herbarium, Department of Botany, University of Rajasthan.

2. To analyze physicochemical constituents of the plant.

3. To purify and identify the isolated component by chromatographic techniques.

4. To study the anti-microbial activity of the essential oils and the isolated phytochemicals by Agar – Diffusion Method and MIC.

REVIEW OF LITERATURE

Trigo *et al* (1988) found several alkaloids which have hepatotoxic activity in the extract of *Ageratum conyzoides*. Elias *et al* (1990) isolated some saponins from *C. officinalis* and reported its antimutagenic activity. Out of more than 2,50,000 species of higher plants, only about 5-10% has been investigated chemically for the presence of biological active compounds (Ayensu and De Filipps, 1978).

According to Kalvatchev *et al* (1997) extracts of dried flowers from *C. officinalis* were examined for their ability to inhibit the human immunodeficiency virus type 1 (HIV-1) replication. Both organic and aqueous extracts were relatively nontoxic to human lymphocytic Molt-4 cells, but only the organic one exhibited potent anti-HIV activity in an in vitro MTT/tetrazolium-based assay. In addition, in the presence of the organic extract (500 micrograms/mL), the uninfected Molt-4 cells were completely protected for up to 24 h from fusion and subsequent death, caused by cocultivation with persistently infected U-937/HIV-1 cells. It was also found that the organic extract from *C. officinalis* flowers caused a significant dose- and time-dependent reduction of HIV-1 reverse transcription (RT) activity. An 85% RT inhibition was achieved after a 30 min treatment of partially purified enzyme in a cell-free system. These results suggested that organic extract of flowers from *C. officinalis* possesses anti-HIV properties of therapeutic interest.

According to Cown (1999), the higher plants have made important contributions in areas beyond anti-infective, such as cancer therapies. According to Iwu *et al*. (1999) the first generation of plant drugs were usually simple botanicals employed in more or less their crude form. Several effective medicines used in their natural state were selected as therapeutic agents based on empirical study of their application by societies from different parts of the world.

Zhang (2000) has estimated that 80 % of the inhabitants of the world rely mainly on traditional medicines for their primary health care needs, and it may be presumed that a major part of traditional healing involves the use of plant extracts or their active principles. According to Fabricant & Farnsworth (2001) the goals of using plants as sources of therapeutic agents are: 1) to isolate bioactive compounds for direct use as drugs; 2) to

produce bioactive compounds of novel or known structures as lead compounds for semi synthesis to produce patentable entities of higher activity and/ or lower toxicity; 3) to use agents as pharmacologic tools; 4) to use the whole plant or part of it as a herbal remedy.

Shahidi and Marian (2003) reported that the differences in antioxidant activities of plant extracts could be due to the variable contents of their phenolic compounds. Lauk *et al* (2003) tested the antibacterial activity of plant part extracts of *C.officinalis* against anaerobic and facultative aerobic bacteria. A method for the efficient preparative purification of faradiol 3-O-laurate, palmitate and myristate, the major anti-inflammatory triterpenoid esters in the flower heads of the medicinal plant *C. officinalis* has been developed. Gram quantities of the individual compounds were obtained with 96 to 98% purity by a combination of supercritical fluid extraction (SFE), normal-phase and reversed-phase column chromatography. During the work-up of the faradiol esters, accompanying minor compounds of the triterpene ester fraction were purified and identified by spectroscopic means as maniladiol 3-O-laurate and myristate. (Hamburger *et al*, 2003).

In *Verbesina encelioides*, terpenoids, sesquiterpenes, flavanol glycosides and triterpenoids have been found to show antiviral, antitumor, hypoglycaemic and anti- implantation efficacies (Jain *et al,* 2007). Del-vechio-vieira *et al*, 2009 concluded that the essential oils of *Ageratum* species were active against *S. aureus, S. mutans, E. coli* but not on *P. aeruginosa* and the yeast *C. albicans* using agar diffusion method and minimal inhibitory concentration. According to BP Muley *et al* (2009) *C.officinalis* exhibits several pharmacological activities such as anti-HIV, anti cancer, anti-inflammatory, hepatoprotective, spasmolytic and spasmogenic. A variety of

phytochemicals such as terpenoids, flavonoids, coumarins, quinones, volatile oil and carotenoids have been reported in this plant.

Chakraborthy *et al* 2010 reported the preliminary phytochemical screening of petroleum ether, chloroform, methanol and water extracts of *C. officinalis* leaves according to which petroleum ether extract showed the presence of fatty acids, chloroform extracts showed the presence of triterpens and sterols. Flavonoids, carbohydrates, amino acids and saponins were present in methanol extract and saponins, phenolic substances and tannins were present in the water extract of *C. officinalis*. From these results it can be depicted that this plant can be used for its various activities like analgesic, immunomodulatory effects, anti-inflammatory and for neurological behavior studies. Mathur *et al* 2011 observed that all the plant part showed significant anti microbial activity but the highest antimicrobial activity was observed in petroleum ether extract of stem part of *C. officinalis* against *E. coli*. Elias *et al* (1990) isolated some saponins from *C.officinalis* and reported its antimutagenic activity. Lauk *et al* (2003) tested the antibacterial activity of plant part extracts of *C. officinalis* against anaerobic and facultative aerobic bacteria. Ethanolic extracts of *V. encelioides* have been found to be effective as a hypolipidemic (Sindhu *et al*, 2011).

In Ageratum conyzoides, certain polyhydroxyflavones like quercetin, kaempferol and their glycosides have been reported to exhibit anti-bacterial, wound healing and gastroprotective activity (Hassan *et al*, 2012). Methanolic extracts from the leaves of A. conyzoides have been reported to show antidysentric and beneficial in treating leprosy, purulent ophthalmia and pneumonia (Zeeshan *et al*, 2012)

METHODOLOGY

To achieve the objectives the work plan is as follows:

Collection and authentication of selected plant species

Sample preparation

Extraction of essential oils by

Clevenger- type apparatus

Soxhlet Extraction for various phytochemicals using solvent series
(n-hexane, chloroform, ethyl acetate, methanol and water)
using Harborne's standard guide

Evaluation of antimicrobial activity
using Agar Diffusion Method and MIC

Identification of compounds by chromatographic techniques
using Thin layer chromatography and Column chromatography

Collection and authentication of selected plant species

Entire plants of *Calendula officinalis*, *Ageratum conyzoides* and *Verbesina encelioides* will be collected and taken for authentication at the Department of Botany, University of Rajasthan

Sample preparation

Stems, leaves and flowers will be separated, shade dried and crushed to powder.

Extraction and physico-chemical analysis

Extraction of solvents from the prepared samples will be done using Soxhlet apparatus. Crushing and grinding of dried sample will be done for extraction and passed through various solvents according to their polarity from non- polar to polar solvents, starting from n-hexane, chloroform, ethyl acetate, methanol and water. The extracted solvent will be subjected to physico-chemical analysis by qualitative and quantitative estimation. The estimated content of the solvent obtained will be calculated as % of the starting materials for quantitative estimation. Qualitative

estimation will be performed by using Harborne's standard guide of phytochemical methods (1998).

Evaluation of antimicrobial activity

The extract will be dissoluted further for microbial analysis using two methods:

Agar Diffusion Method

The agar diffusion test, also known as Kirby-Bauer disk-diffusion method, is a means of measuring the effect of an antimicrobial agent against bacteria grown in culture. The bacterial culture is swabbed uniformly across a culture plate. A filter-paper disk, impregnated with the compound to be tested, is then placed on the surface of the agar. The compound diffuses from the filter paper into the agar. The concentration of the compound will be highest next to the disk, and will decrease as distance from the disk increases. If the compound is effective against bacteria at a certain concentration, no colonies will grow where the concentration in the agar is greater than or equal to the effective concentration. This is the zone of inhibition. Thus, the size of the zone of inhibition is a measure of the compound's effectiveness: the larger the clear area around the filter disk, the more effective the compound.

Minimal Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration (MIC) is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. Minimum inhibitory concentrations are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents. A lower MIC is an indication of a better antimicrobial agent. A MIC is generally regarded

as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism.

Chromatographic techniques

To identify and isolated the obtained compounds using Thin layer chromatography and Column chromatography.

SIGNIFICANCE

It is widely accepted that research on medicinal plants should be focussed primarily on species whose pharmaceutical activities have already been demonstrated. The present study is therefore designed to provide scientific evidence for the selected plants to be used as a traditional folk remedy by investigating the phytochemical constitution of the plant as well as the antibacterial activity. Essential oils would be evaluated for antimicrobial activity against gram- positive and gram- negative bacteria strains obtained from standard agencies.

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