Phytochemical Screening, Antimicrobial & Anti Proliferative Properties of Centipeda minima (Asteraceae) on Prostate Epithelial Cancer Cells

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ABSTRACT

Natural products continue to play an important role in the discovery and development of new biologically interesting molecules. Several chemical compounds have been extracted and identified from its species known as Centipeda minima (L) (Asteraceae). The present study is designed for phytochemical analysis of Centipeda minima and extraction of bioactive compound by HPLC, further antimicrobial activity of the bio active compounds has been investigated by crude and the column extract and antiproliferative activity on prostate epithelial cancer cell (PC3). Our study revealed the presence of the bioactive component plenolin of this plant has good antimicrobial activity and also exhibiting anti cancer properties.

Key words: Phytochemical Screening, Antimicrobial activity, Anti proliferative, Prostate Cancer Cells (PC3), Centipeda minima.

INTRODUCTION

Centipeda minima (Asteraceae) is an annual herbaceous plant found in moist places. However, the widespread use of antibiotics in human medicine and agriculture has caused serious problem of bacterial resistance (Becovi et al., 2006). Therefore, plant derived antimicrobial agents with high potency and low mammalian toxicity, useful for food preservation and human health, have gained special interest in recent decades (Smid, E. J.& Gorris, L. G M (1999), & Reische et al., 1998). Recent pharmacological interest has focused on its anti-allergy and antibacterial effects (Wu et al., 1991). The aerial parts of the C. minima are used to treat headaches, head colds, conjunctivitis, piles and malaria (Perry, 1980). Phyto chemical studies of its composition have led to the identification of a number of terpenes, including sesquiterpene lactones and triterpenes (Wu et al., 1991, Bohlmann, F. & Chen, Z.L. (1984)). The former class contained the major active constituents contributing to the anti-allergy and anti-bacterial activities of the herb (Essawi, T & Sourr, M (2000)). Despite it is used in the Chinese folk medicine to treat naso pharyngeal carcinoma (NPC) (Cheng, J.H & Li (1998) & Zhang., (2000)). Besides, both the anti-nasopharyngeal carcinoma potential and the potent constituents of C.minima remain elusive. Sesquiterpene lactones, most widely distributed within the Compositae, have received considerable attention for their anticancer properties (Zhang et al., 2005). 6-O-angeloylenolin, a sesquiterpene lactone containing an, ß-unsaturated cyclopentenone, isolated from C. minima was reported to induce apoptosis in HL-60 cells in vitro and inhibit the solid cancer growth in Lewis lung cancer xenograft model (Li et al., 2008).

In the present investigation, we have analyzed the bioactive compounds available in the plant Centipeda minima by HPLC. Further, anti-bacterial activity of the crude and column extractions of the active component & Anti proliferative activity of component on prostate epithelial cancer cells (PC3).

MATERIALS AND METHODS

Centipeda minima plants were collected from Botanical garden, India and plant species was identified and confirmed by taxonomist.

Extraction and Analysis

Fresh flowers, stem, root & leaves were collected, washed and weighed (10 g each). The materials were then macerated in 10 ml of water, methanol, acetone & benzene separately and then kept for 6 h at room temperature. The mixtures were then filtered through sterile Whatman filter paper and filtrates obtained were then centrifuged at 5000 rpm for 5 min. The supernatants were collected in a fresh beaker and allowed it to evaporate. Then the dried extracts were stored at 4°C. For working these extracts were dissolved in 1-3 ml (w/v) of dimethyl sulfoxide (DMSO) (Priya and Ganjewala 2007) and processed for further analysis. The pure bioactive compounds from the above extracts (flowers, stem, roots & leaves) were obtained by passing through the silica gel column, activated charcoal and again silica gel in ratio of 1:2:1. Thus the collected fractions were passed through the column, several times, to obtain the pure compound.

Phytochemical Screening

Specific qualitative tests were performed for detection of metabolites in leaf and flower extracts. Alkaloids were estimated as described by Clarke and Williams 1955. The presence of sterols was confirmed by the addition of 2 ml of acetic anhydride to 0.5 g of dried ethyl acetate extract with 2 ml of concentrated sulphuric acid. For the identification of phenolics equal volume (1 ml) of neutral ferric chloride and extract were added. For the identification of terpenes, the extracts were treated with tin and thionyl chloride. For the identification of Flavones, 10 % NaOH was added. To confirm the presence of tannins, 0.5 g of dried powder of leaves and flowers were boiled in 5 ml of water in a test tube and then filtered. To the filtrate, ferric chloride was added and kept undisturbed for the observation. Phospholipids and glycolipids were estimated according to Roughan and Batt 1969, Lowry and Tinsley 1976. To reveal the presence of Fixed oils, small quantity of petroleum ether and benzene extract was pressed separately between two filter papers.

HPLC Analysis

The compounds that are obtained from the column clean up from aqueous extract, leaf in acetone, methanolic extract and flower in acetone extracts was set up for HPLC (High performance liquid chromatography) to test the purity of the compound. We have used reverse phase HPLC Phenomenex C18 column and a Rheodyne injection valve with a 20 µl fixed sample loop.
A binary solvent system Na2HPO4 (90%) and acetonitrile (10%) was used as the mobile phase with a flow rate of 1 ml/min. Equilibration is done before the run of the sample. The eluant was monitored using UV detector at 400 nm. The gradient program was set up and the peak analysis was estimated by observing the graph and comparing the obtained chromatogram with that of the already available data. (Plumb et al., 2004).

**Anti Bacterial Activity**
The bacterial strains Escherichia coli (MTCC 46), Pseudomonas aeruginosa (MTCC 4727), Bacillus subtilis (MTCC 1133) and Streptococcus aureus (MTCC 7443) were collected from microbial type culture collection (MTCC) of IMTECH, Chandigarh.

Pure cultures were maintained by taking a loopful of culture from stock cultures and it was streaked on Petri plates by streak plate method to obtain fine isolated colonies. The plates were incubated at 37°C for 24 hrs. Pour plates allowed for the growth of isolated colonies on agar. Agar well diffusion method (pour plate method), was followed to test the antibacterial activity by Deena and Thoppil 2000. The inoculum containing the microorganism from the broth was poured on a sterile agar medium plate. Again the loop was sterilized on the flame and continued to pour the bacteria again. The plate was rotated 90°C and the bacteria were spread. The process was repeated as per the requirement. Later the plates were incubated. The crude and column cleaned up HPLC compound were used to test the antibacterial activity by disc diffusion method. The plates were observed for inhibitory activity of tested chemical group on culture growth.

**Anti Cancer Activity**

**Cell Lines and culture conditions**
The anticancer activity of the extracted compounds was studied by the cell viability and morphological analysis. Human Prostate Aden carcinoma epithelial cell lines (PC3) were collected from National centre of cell science, India and routinely cultured in Dulbecco modified eagle’s medium. Culture medium was prepared by taking precautions of bacterial and fungal contaminants by changing volume of trypsin / EDTA we can bring the cultures cell lines in to adherent and semi adherent suspensions and they are used directly. Aliquot of cells (100-200µl) are removed and performed cell count and the cell viability should not excess 90% in achieve good recovery after freezing and they resuspended in to freezing medium.

**Cryopreservation of Cell line**
Cultures were observed using an inverted microscope to assess the degree of cell density and confirm the absence of bacterial and fungal contamination adherent and semi adherent cells were brought in to suspension using trypsin / EDTA and were resuspended in a volume of trypsin. Suspension cell lines can be used directly.

**Cell Treatment**
Centipeda minima extracted compounds obtained by column clean up were used to evaluate anticancer effect. These compounds were prepared as 10 mM stock solution in 100 % DMSO and were stored in dark color bottle at 40C. The cells were exposed to drug individually for a period of 48 hrs. Cells grown in the medium containing equivalent amount of DMSO without drug serve as control.

**RESULT AND DISCUSSION**
The appearance of pink color when crude extract is treated with tin and thioynl chloride indicates the presence of terpenes. When crude extract is treated with 10% NaOH shows change in color from yellow to orange indicates Flavones. Oil stained on paper when benzene extract treated with petroleum ether indicates the presence of fixed oil. Appearance of cream color when treated with Mayer’s reagent indicates the presence of an alkaloid (Table 1).

**Table 1: Phytochemical screening of Centipeda minima extracts.**

<table>
<thead>
<tr>
<th>Qualitative Test</th>
<th>Centipeda minima</th>
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<tbody>
<tr>
<td>Terpenes</td>
<td>+++</td>
</tr>
<tr>
<td>Fixed Oils</td>
<td>+</td>
</tr>
<tr>
<td>Flavones</td>
<td>+++</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>++</td>
</tr>
<tr>
<td>+: Low, ++: Medium &amp; +++: High concentration.</td>
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</table>

**HPLC Analysis**
The column cleaned up compound was analyzed with the HPLC and the compound was plenolin (Figure 1), of peak height 6.5871. This plenolin was used for testing antibacterial activity.

**Figure 1: HPLC Analysis of Centipeda minima extracts showing the peaks of active component.**

**Anti-Bacterial Activity**

**C.minima** flower extracts possess strong antibacterial activities compared to corresponding leaf extracts. Extracts were prepared in acetone, methanol and water. C. minima crude extracts of flower and leaf showed the highest inhibitory effects against B. subtilis with a measured value of zone of inhibition area ranging from 6-9.2 mm. Column extracts compared to the crude extracts, displayed less inhibitory effects against all the bacteria tested with a relatively smaller zone of inhibition area ranging from 3.3-6.6mm. E. coli was found to be the most sensitive bacteria to all C.minima column and crude extracts. P. aeruginosa and E.fecalis was also found to be highly susceptible to all C.minima crude and column extracts.

The order of susceptibility of plenolin isolated from Centipeda minima on microorganisms is as follows: Paeruginosa > B.subtilis > S.aureus> E .coli( Table 2).

**Table 2: Anti-Bacterial activity of crude and column extracts Centipeda minima.**

<table>
<thead>
<tr>
<th>Microbes</th>
<th>Zone of Inhibition in mm</th>
<th>Column</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Crude</td>
<td></td>
</tr>
<tr>
<td>E.coli</td>
<td>6.9±0.6</td>
<td>4.5±0.6</td>
</tr>
<tr>
<td>S.aureus</td>
<td>7.2±0.4</td>
<td>4.1±0.4</td>
</tr>
<tr>
<td>B.subtilis</td>
<td>7.1±0.8</td>
<td>5.8±0.8</td>
</tr>
<tr>
<td>Paerugen</td>
<td>8.0±0.6</td>
<td>5.5±0.8</td>
</tr>
</tbody>
</table>

Phytochemical studies on C. minima were reported, they have more than ten sesquiterpene lactones which are all pseudo-guanianolide or guaianolide types (Taylor et al., 1998). Sesquiterpenoids were also found to have antagonistic activities for platelet activating factor and antibacterial activities (Iwamaki et al., 1992). The plenolin and helanolin have same activity against
the Bacillus and Sreptococcus species (lee et al.,1977). Bioactive potential of Flavonoids has been reported. (Ilic et al.,2004, Cushner and Lamb 2005). The present results are compared with Samy and Ignace Muthus (1999) & Sanchez et al (2005), sesquiterpenes are considered to be active components of this plant (Wu et al.,, 1985). Based on the fact that microorganism are becoming resistant against the drugs in use, present investigation is of great importance in pharmaceutical industries for preparing plant based antimicrobial drugs.

AntiCancer Properties
From the above figures 2(a),3(a),4(a) & 5(a) represents the control of cells have only prostate cancer (PC3) cell lines. The fig 2(b) represents the leaf extract in acetone exhibiting more anticancer properties than stem in water (4(b), Flowe in water (3(b)) and Root in water (5(b)).

Table 3: Prostate cancer cell (PC3) death percentage of Centipeda minima.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Sample</th>
<th>Solvents</th>
<th>Cell Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td></td>
<td>100%</td>
</tr>
<tr>
<td>2</td>
<td>Root</td>
<td>Water</td>
<td>10.49%</td>
</tr>
<tr>
<td>3</td>
<td>Stem</td>
<td>Water</td>
<td>9.86%</td>
</tr>
<tr>
<td>4</td>
<td>Leaf</td>
<td>Acetone</td>
<td>89.18%</td>
</tr>
<tr>
<td>5</td>
<td>Flower</td>
<td>Water</td>
<td>9.76%</td>
</tr>
</tbody>
</table>

Fig: 2: Control (a) compared with leaf extract (b) with acetone in PC3 cells

Fig: 3: Control (a) compared with flower (b) in water in PC3 cells

Fig: 4: Control (a) compared with stem (b) in water in PC3 cells

Fig: 5: Control (a) compared with root (b) in Water in PC3 cells.

The reduction in overall tumor incidence can be attributed to ability of these compounds to interfere in the initiation of carcinogenesis and thereafter promotion of tumors (Jagdeep et al.,2008). The present results are agreement with that of I. viscose (Compositae) leaf extract induced cytogenetic alterations (cytoplasmic shrinkage, nuclear condensation, DNA fragmentation, membrane blebbing, cytoskeleton alterations and appearance of apoptotic bodies) aid mainly cell death in root tips of A. cepa (liliaceae) (Tulay et al.,2010). The present results are useful to develop new molecules for clinical/pharmalogical benefits in a variety of clinical disorders.

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REFERENCES


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