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PHYTOCHEMICAL SCREENING, ANTIMICROBIAL & ANTI PROLIFERATIVE PROPERTIES OF CENTIPEDA MINIMA 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 pharmaceuticals. Several chemical compounds have been extracted and identified from its species known as *Centipeda minima (C.minima)*. The present study is designed for phytochemical analysis of *Centida minima* and extraction of bioactive compound by HPLC, also includes the antimicrobial activity of the bio active compound obtained by crude and the column extract and antiproliferative activity on prostate epithelial cancer cell (PC3). The study had shown the presence of the bioactive component plenolin of the plant has been showing good antimicrobial activity and plant also exhibiting anti cancer properties.

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

No. of Tables: 3 No. of Figures: 5 References: 26

INTRODUCTION

Centipeda minima (Compositae) 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 (Beovic 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).phytochemical 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 & Srour, 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, β-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 report the analysis of bioactive compounds available in the plant *Centipeda minima* by HPLC, anti-bacterial activity of the crude and column extractions of the active component & Anti proliferative activity on prostate epithelial cancer cells (PC3).

Materials and Methods

Centipeda minima plants were collected from botanical garden, Hyderabad and plants identity was confirmed by plant 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 Whatmann filter paper No.1. The filtrates obtained were then centrifuged at 5000 rpm for 5 min. The supernatants were collected in a beaker and the solvents were allowed to evaporate. Then the dry extracts were stored at 4°C. These extracts were dissolved in 1-3 mL (w/v) of dimethyl sulfoxide (DMSO) (Priya and Ganjewala 2007). The samples were further extracted by passing through the column of cotton, silica gel, activated charcoal and again silica gel in ratio 1:2:1 to obtain the extracts. Thus, the collected extract was passed into the column, number of 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 from previously published procedures (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, one ml of neutral ferric chloride was added to one ml of the extract. For the identification of terpenes, the extracts were treated with tin and thionyl chloride. For the identification of Flavones, 10 % sodium hydroxide was added. To reveal the presence of tannins, 0.5 g of the dried powder of the leaves and flowers were boiled with 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 based on previously published procedures (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 column cleaned compounds, *Centipeda minima* extracts (Root, Stem, Leaf & Flower), were tested for the compound conformity and purity in the HPLC mobile and stationary phases were used for testing. 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

Pour Plate Technique

Nutrients required for the growth of micro-organisms were taken into a 250ml conical flask and 100 ml of distilled water was added. pH was adjusted to 7.2 and 2 gm of agar was added. Then the nutrient agar medium was sterilized in an autoclave at 121°C under 15 lbs pressure for 15-20 minutes.

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

Pure cultures nutrient agar plates were prepared by taking a loopful of culture from stock cultures and it was streaked on Petri plates in streak plate method to obtain fine isolated colonies. The petri plates were incubated at 37°C for 24 hrs.

Pour plates allow for the growth of isolated colonies on the surface of the agar. The standard procedure, agar well diffusion method, was followed to test the antibacterial activity by pour plate method (Deena and Thoppil 2000). A loopful of inoculums 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 for about 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 method. The plates were observed for inhibition of culture growth by the tested compounds.

ANTI CANCER ACTIVITY

Cell Lines and culture conditions:

The anticancer activity of the extracted compound was studied by the cell viability and morphological analysis. Human Prostate Aden carcinoma epithelial Cancer cell line (PC3) were collected from National centre of cell science, pune and routinely cultured in *Dulbecco modified eagles* medium. Culture medium preparation was carried out by taking precautions of bacterial and fungal contaminants. By changing volume of Trypsin/EDTA we can bring the cultures cell lines into 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 observe 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 into 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 10mM stock solution in 100 % DMSO and were stored in dark color bottle at 4^0C. The cells are 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

Table 1: Phyto Chemical screening of *Centipeda minima*

Qualitative Test	*Centipeda minima*
Terpenes	+ ++
Fixed Oils	+
Flavones	+++
Alkaloids	+ +

+: Low, ++: Medium & +++: High concentration

The pink color appearance when crude extract is treated with tin and thionyl chloride indicates the presence of terpenes. The change in color from yellow to orange when the crude extract is treated with 10% NaoH shows the presence of 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).

HPLC Analysis

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

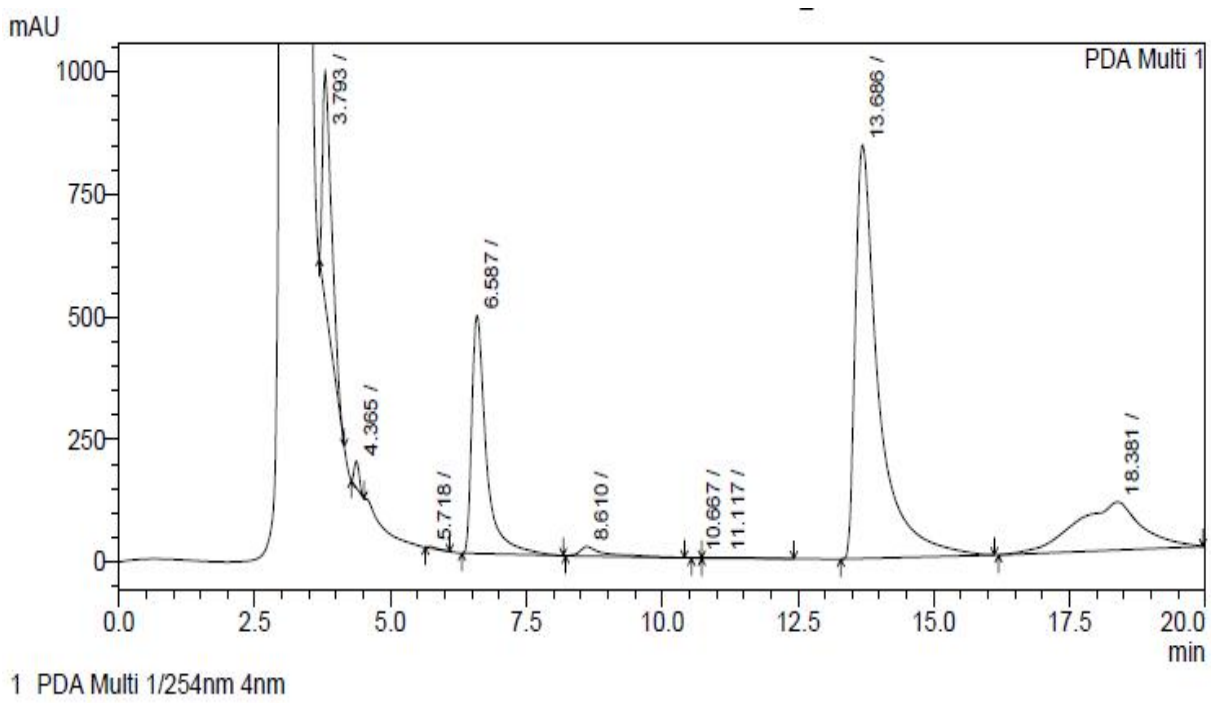


Figure 1: HPLC Analysis of *Centipeda minima* extracts showing the peaks of active component**.**

Anti-Bacterial Activity

C.minima flower extracts possess strong antibacterial activities more than the 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. subtillis* 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.

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

Microbes	**Zone of Inhibition in mm Mean ± SD**	
	Crude	**Column**
E.coli	7.6±0.6	4.4±0.6

S.aureus	7.7±0.8	4.5±0.2
B.subtilis	7.2±1.2	5.8±0.8
P.aerugens	8.4±0.4	5.6±0.8

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

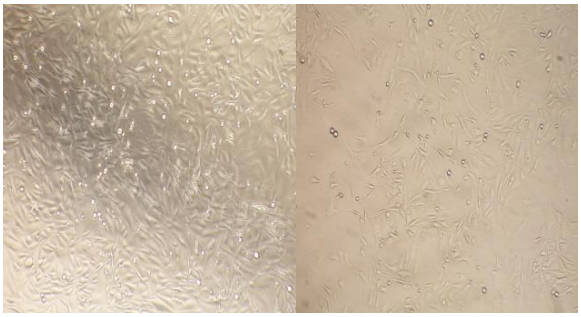
Previous phytochemical studies on *C. minima reported* that they *have* more than ten sesquiterpene lactones which are all pseudo-guanianolide or guaianolide types (Taylor et al.,1998). Sesquiterpenoids were also found to have antagonistic activities for platelet activating factor and antibacterial activities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

AntiCancer Properties:

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

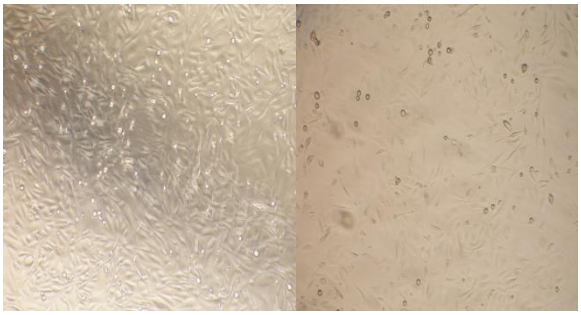
S. No	Sample	Solvents	Cell Death
1	Control		100%
2	Root	Water	8.49%
3	Stem	Water	7.86%
4	Leaf	Acetone	91.18%
5	Flower	Water	7.76%

Fig: 2: Control compared with leaf extract with acetone in PC3 cells



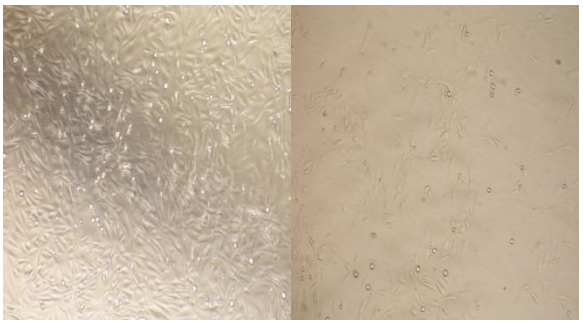
2. (a) 2.(b)

Fig: 3: Control compared with flower in water in PC3 cells



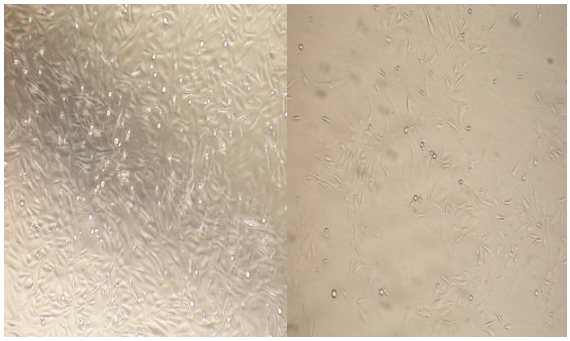
3.(a) 3.(b)

Fig: 4: Control compared with stem in water in PC3 cells.



4.(a) 4.(b)

Fig: 5: Control compared with root in Water in PC3 cells.



5. (a) 5.(b)

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), Flower in water (3(b)) and Root in water (5(b)).

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 comparable to that of I. *viscosa* 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 (*Tulay et al.,2010). The present results are useful for designing novel therauptic agents.

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