

Full Length Research Paper

Isolation of cannabinoids from the plant *cannabis sativa* L and its potential anticancer activity

Tariq A.L¹ and Reyaz A.L²

¹Department of Microbiology Sree Amman and Science College Erode-638102

²Department of Biotechnology Periyar University Salem-636011

Accepted 06 February, 2012

The plant leaves were identified as *Cannabis sativa* L. The cannabinoids were extracted by aqueous extract found a total yield of 3.8g while as acetone extract 4.8g. The protein content in crude extract of *Cannabis sativa* L for aqueous extract found 112µg/ml and for acetone extract 160µg/ml. The molecular weight of protein by SDS PAGE found to be 70KDa. The HPLC intensity percentage for aqueous was 11 while for acetone extract it found 25. The acetone extract exhibited more anticancer activity against HT29, MCF7 and SF-26 Cells

Keywords: Cannabinoids, 70KDa protein, Acetone extract, cell line culture potential anticancer activity,

INTRODUCTION

The applications of cannabinoids have been known to exert palliative effects in cancer patients (Bifulco et al., 2007). Cannabinoids are proposed as potential antitumoral agents on the basis of experiments performed both in cultured cells and in animal models of cancer (Guzmán, 2003). It is broadly used as medicine in China, India, Southeast Asia, South Africa, South America for malaria, rheumatic pains, constipation and childbirth (Pagotto et al., 2006). Cannabinoids have anti-inflammatory and antinociceptive properties that involve receptor dependent and independent mechanisms (Pertwee, 2001).

Cannabis sativa (Bhang) is a major constituent of the Jatiphaladya commonly grown herb of high medicinal importance (Kumar et al., 2001). The proapoptotic and antineoplastic properties of cannabinoids shows emphasis effects of *N*-acylethanolamines (Pushkarev et al., 2008; Regina, 2008; Dubravka et al., 2006). Cannabinoids present an interesting therapeutic potential such as antiemetics, appetite stimulants in debilitating diseases, analgesics, treatment of multiple sclerosis, spinal cord injuries, Tourette's syndrome, epilepsy and glaucoma (Mohamed Ben Amar, 2006). The dronabinol has an anti-depressive potential that can readily be used

in medical practice (Kurt, 2008). A simple and reliable thin layer chromatographic method is used in a micellar solution of sodium dodecyl sulfate as mobile phase for the identification of all the four herbal drugs with preliminary separation on silica gel 'G' TLC plate (Armentano, 2011; Ligresti et al., 2006).

MATERIALS AND METHOD

Collection and preservation of specimen:

The plant leaves of *Cannabis sativa* were collected from Kolli hills, Tamil Nadu and were taken to the laboratory; subsequently they were preserved in 10% neutralized formalin at low temperature.

Extraction of crude toxin:

The aqueous extract of sample was prepared by (Guzman, 2003) squeezing the sand free specimens in triple distilled water. The resultant solution was filtered and dialyzed by using sigma dialysis membrane-500 (Av Flat width-24.26 mm, Av. Diameter-14.3mm and capacity approx-1.61ml/cm) against D-glucose to remove the excess water. The supernatant so obtained stored at 4°C and then used as crude aqueous extract.

*Corresponding author E-mail: tariqtasin@gmail.com

Acetone extraction

The sample was dried in air for 2 days and after complete drying, crushed in the form of fine powder then 10g sample was added into 200ml of acetone, covered and allowed to stand for 5 hours (Teske et al., 2002). The solvent was then removed after squeezing the sample and filtered through Whatman filter paper No1. The solvent was evaporated at low pressure by using a Buchi Rotavapor R-200 at 4°C and stored in refrigerator to use as crude acetone extracts.

Purification of Crude Protein

Purification of the crude extract was carried out Teske et al., (2002) by using DEAE cellulose anion exchange chromatography equilibrated with 10mM Tris-HCl buffer pH 8.3. The 15ml of crude extract was eluted with 10mM Tris-HCl buffer pH 8.3 at the flow rate of 20ml/h. A linear gradient consisting of 50ml of 10mM Tris-HCl buffer pH 8.3 and 50ml of the same buffer with 0.3M NaCl. The 5ml of fractions elute was collected and absorbance measure at 280nm.

Protein Estimation

Protein estimation of *Cannabis sativa L* extracts was done as described by Lowry et al. (1951) by using Bovine serum albumin standard and absorbance was read at 650nm under spectrophotometer.

Characterization of protein

Sodium Dodecyl Sulphate–PAGE

One dimensions of sodium dodecyl sulphate polyacrylamide gel electrophoresis was carried out following the modified method of Lammelli et al., (1970). The molecular weight of the protein determined by staining the protein with 10% methanol, 7% acetic acid and 0.2% coomassie brilliant blue for 4 hours and destained with 10% methanol, 25% acetic acid solution for 12 hours.

HPLC Chromatography

The column used here by following Debruyne et al., (1999) was “Normal Phase” having Cartridge 8 x 100 mm Bondapak C18 and Solvent contains 0.5% phosphoric acid in 40% aqueous acetone. In HPLC, narrow columns with internal diameters 2-80 mm was used. The solvents was filtered through fine membranes (0.4-0.5 micron) and all solutions were injected into the Waters instrument

comprises of three main components: the injector (located on the top right-hand corner of the console), the solvent delivery system (right-hand side) and the UV detector (left-hand side).

Source and Maintenance of Cell Lines

Cells lines representing the most common human cancers (WHO, 2006) were obtained from the National Cancer Institute (NCI). The cell lines include the breast adenocarcinoma (MCF-7), the glioblastoma (SF-268) and the colon adenocarcinoma (HT-29). The three cell lines (Massi et al., 2004) were cultured in 200 ml flasks (Nunc) with the complete culture medium (RPMI-1640 or DMEM). The cells were maintained at 37 °C in a 5% CO₂ humidified incubator. The culture medium (20 ml) was replaced three times weekly and the cells were trypsinised when nearly confluent.

Preparation of cell suspension

All the cells were detached from the culture flasks by addition of 1 ml of 0.25% trypsin-0.1% EDTA (Massi et al., 2004). Trypsin was then inactivated by the addition of 10 ml of experimental medium (RPMI-1640 or DMEM). The cells were separated into a single cell suspension by a gentle pipetting action and 1 ml of the cell suspension was returned to the culture flask containing 20 ml of culture medium. The remaining cell suspension was centrifuged at 1000 rpm (Sorvall® T6000D) for 3 minutes and the supernatant discarded. Experimental medium (20 ml) was added to the cells which were then repeatedly pipetted to allow cells to disperse in to the medium. An aliquot of the trypsinised cell suspension was stained with 2 mg/ml trypan blue (1:1 ratio) in order to obtain a more quantitative analysis of the cell suspension. Trypan blue is a water-soluble dye, which is insoluble in the cell membrane lipids. It will thus only cross cell membranes of dead/non-viable cells. On visualization with the light microscope, viable cells appear transparent, while nonviable cells are stained dark blue. The haemocytometer was used to determine the total number of viable and non-viable cells by counting cells in the 25 squares (each square is subdivided into 16 smaller squares of 0.1 mm²) at the top and bottom of the haemocytometer and the average number of cells per unit volume (millimeter, ml) of medium calculated. The cell suspension was adjusted with experimental medium to approximately 1.5 x 10⁵ cells /ml. The experiment was carried out only when there was at least 95% cell viability.

Percentage cell viability was calculated as follows

% Cell viability = $\frac{\text{Abs test sample} - \text{Abs background}}{\text{Abs test sample} - \text{Abs background}}$



Figure 1. Morphological appearance of Leaves showed that belongs to Kingdom-Plantae, Class-Magnoliopsida, Order-Rosales, Family-Cannabaceae, Genus-Cannabis, Species-Sativa L

sample–Mean Abs blank x100 (Eq. 10.1)

Mean Abs control– Mean Abs blank

Where: Abs: absorbance at 492 nm

RESULT

Identification of Leaves

The present study deals with to study the anticancer activities of herbal plant *Cannabis sativa L*. The sample collected from Kolli hills, Tamil Nadu, was identified based on the taxonomical characters (Figure 1). Kingdom-Plantae, Class-Magnoliopsida, Order-Rosales, Family-Cannabaceae, Genus-Cannabis, Species-Sativa L

Extraction of Protein

Acetone extract of plant sample *Cannabis sativa L* yielded a total amount of 3.2g of crude extract from 500g of sample. Similarly from aqueous extract at a total amount of 4.8g of crude extract was yielded.

Protein Content

The optical density values of aqueous extract obtained 0.530nm which was corresponds to 0.6mgg/ml. While in acetone extract the optical density found 0.676nm which corresponds to 1.6mg/ml (Table 1).

DEAE Chromatography

The optical density value of cannabinoid of aqueous extract at 280nm was 1.530nm and for acetone extract the optical density of cannabinoid was 1.745nm.

Molecular weight of Protein

Sodium dodecyl sulphate polyacrylamide gel electrophoresis on 12% gel, the crude protein toxins yielded 3 bands in aqueous extract and 5 bands in acetone extract of *Cannabis sativa L* with one well defined band of 70 kDa (Figure 2).

HPLC chromatography

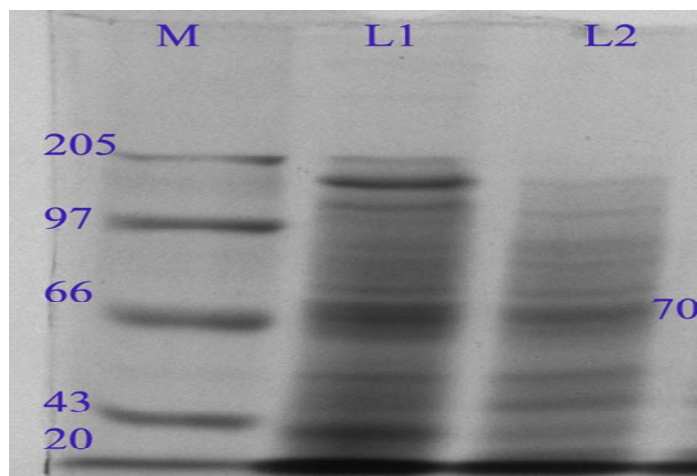
Under the high performance liquid chromatography it was analyzed that in the acetone extract of retention time of 3.27min the percentage intensity was found to be 11 for aqueous extract and 25 for acetone extract. In the aqueous extract the retention time of 4.75min, the percentage intensity was found to be 32 for aqueous and 30 for acetone extract.

Anticancer activity

The aqueous extract exhibited anticancer activity against HT-29, MCF-7 and SF-268 cell lines. It showed potential anticancer activity as the concentration was increased

Table 1. Estimation of protein by acetone and aqueous solvents.

S.No	Type of Extract	Conc of protein (mg/ml)
1.	Acetone	1.6mg/ml
2	Aqueous	0.6mg/ml

**Figure 2.** Determination of molecular weight of proteases by sodium dodecyl sulphate agarose gel electrophoresis.

M— Molecular marker mass standards: phosphorylase b (205 kDa), tyrosine (97kDa), acid phosphate (66kDa), glutamic dehydrogenase (43 kDa) and aldolase (20 kDa)

L1—Acetone extract of Cannabinoids showed 5 bands with distinct band of 70KDa.

L2—Aqueous extract of Cannabinoids showed 3 bands with distinct band of 70KDa

which lead the reduction of Trypan blue that means inhibited the proliferation of cancer cells and the background appeared transparent. In acetone extract which showed more anticancer activity against activity against HT-29, MCF-7 and SF-268 cell lines. It showed potential anticancer activity as the concentration was increased which lead the reduction of Trypan blue that means inhibited the proliferation of cancer cells and the background appeared transparent (Figure 3).

DISCUSSION

It is noteworthy that most of the CB1 receptor antagonists developed to date, including rimonabant, has inverse agonist properties, so their effects do not necessarily reflect reversal of the tonic action of the endocannabinoids (Osch S and Gertsch J 2009). Rimonabant counteracts the anti-tumour effects of anandamide-related compounds and other cannabinoid agonists in thyroid, breast and prostate cancers. Unexpectedly, rimonabant per se showed a potential anti-

tumour action in thyroid, mantle-cell lymphoma and breast tumours both in vitro and in vivo (Bifulco et al., 2007). Cannabinoids in cancer therapy as palliative agents the potential application of cannabinoid agonists as anticancer agents is still at the preclinical level. Meanwhile the cannabinoids are emerging as valuable adjunctive agents for optimizing the management of multiple symptoms of cancer and the treatment of therapy-related side-effects (Mackie K 2006, Hall et al., 2005). Regarding effectiveness, cannabinoids exert notable antitumour activity in animal models of cancer, but their possible antitumour effect in humans has not been established. Regarding toxicity, cannabinoids not only show a good safety profile but also have palliative effects in patients with cancer, indicating that clinical trials with cannabinoids in cancer therapy are feasible (Massi P 2004, Amtmann et al., 2004). As cannabinoids are relatively safe compounds, it would be desirable that clinical trials using cannabinoids as a single drug or in combined anticancer therapies could accompany these laboratory studies to allow us to use these compounds in the treatment of cancer. Ligresti et al (2006).

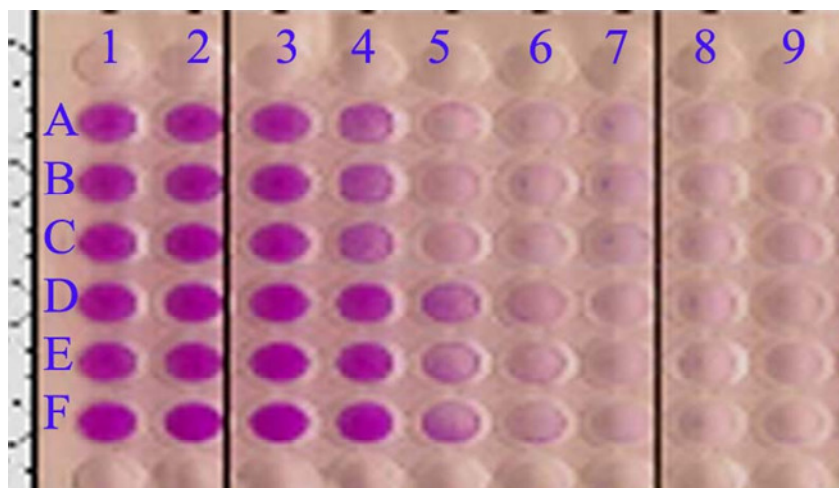


Figure 3. MTT analysis assay of cannabinoids against HT-29 cells, MCF7-cells, SF-26 cells

I. Where A1, A2 are blank controls and A3 to A9 are various aqueous concentration of cannabinoids in microtitre wells containing of HT-29 cell. Where B1, B2 are blank controls and B3 to B9 are various aqueous concentration of cannabinoids in microtitre wells containing of HT-29 cell. Where C1, C2 are blank controls and A3 to A9 are various aqueous concentration of cannabinoids in microtitre wells containing of HT-29 cell.

II. Where D1, D2 are blank controls and D3 to D9 are various acetone concentration of cannabinoids in microtitre wells containing of HT-29 cell. Where E1, E2 are blank controls and E3 to E9 are various acetone concentration of cannabinoids in microtitre wells containing of HT-29 cell. Where F1, F2 are blank controls and F3 to F9 are various acetone concentration of cannabinoids in microtitre wells containing of HT-29 cell.

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