

## EVALUATION OF ANTIPROLIFERATIVE POTENTIAL OF *MYRMECODIA PENDANS* AND ITS ACTIVITY ON IL-8 SECRETION IN COLON CANCER CELL

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Manuscript received: January 2020

### Abstract

*Myrmecodia pendans* (*M. pendans*, *Rubiaceae* family) is a plant with multiple biological properties due to its active compounds. Although it is successfully used in the origin area, in various types of diseases (from infectious disease to diabetes or cancer), just a few studies have analysed its chemical content and its antitumour effects. The present study reports the antiproliferative biological activities of aqueous and ethanolic extracts of *M. pendans* in colon cancer cells (HCT116) associated with interleukin 8 (IL-8) secretion. Tumour cells were incubated with different concentrations of extract (between 150 and 600 µg/mL) for 24, 48 and 72 h. Cellular metabolic activity was measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay. The IL-8 secretion was analysed using the Elisa method. Both extracts exhibited moderate antitumour activity, dose and time dependent in cells incubated with the ethanolic extract 24 and 72 h, with IC50 value of 566.087 µg/mL at 72 hours incubation. The exposure of the cells to aqueous extracts decreased the rate of cell multiplication, dependent on dose and incubation time. The IC50 was 587.95 µg/mL, and 512 µg/mL respectively for a period of 48 and 72 h incubation. The extracts of *M. pendans* did not influence IL-8 levels in cell cultures.

### Rezumat

*Myrmecodia pendans* (*M. Pendans*, *Rubiaceae*) este o plantă cu multiple proprietăți biologice datorită compușilor săi activi. Deși este utilizată cu succes în zona de origine, în diferite tipuri de boli (de la boli infecțioase la diabet sau cancer), doar puține studii au analizat conținutul său chimic și efectele sale antitumorale. Prezentul studiu raportează activitățile biologice antiproliferative ale extractelor apoase și etanolice ale *M. pendans* în celulele cancerului de colon (HCT116) asociate cu secreția de interleukină 8 (IL-8). Celulele tumorale au fost incubate cu concentrații diferite de extract (între 150 și 600 µg/mL) pentru 24, 48 și 72 ore. Activitatea metabolică celulară a fost măsurată prin testul colorimetric MTT. Secreția IL-8 a fost analizată prin metoda Elisa. Ambele extracte au prezentat o activitate antitumorală moderată, dependentă de doză și timp în celulele incubate cu extract etanolic la 24 și 72 ore, cu o valoare IC50 de 566,087 µg/mL la 72 ore de incubare. Expunerea celulelor la extracte apoase a scăzut viteza de înmulțire a celulelor, dependentă de doză și de timpul de incubare. IC50 a fost 587,95 µg/mL, respectiv 512 µg/mL pentru o perioadă de incubare de 48 și 72 ore. Extractele de *M. pendans* nu au influențat nivelul IL-8 în culturile celulare.

**Keywords:** antiproliferative, ethanolic and aqueous extracts, HCT116 cells, IL-8, *Myrmecodia pendans*

### Introduction

Phytotherapy is the oldest therapy used in the world; all ancient peoples had extensive knowledge of empirical phytotherapy, the plants being used as a source of food for people but also as a source of medicines and healing drinks. Beginning with a flavonoids mixture from the leaf of *Acinos alpinus* [1], allicin from garlic and tyramine from mistletoe [2], capsaicin from

chili peppers [3], 6-, 8- and 10-gingerols from ginger [4], and different pentacyclic terpenoids from birch bark [5], all of these phytochemicals have been used for millennia.

Colon cancer incidence rate and mortality represent nowadays a serious public health issue in the world as it is increasing at variable rates in population. Treatment with chemotherapeutic agents may cause

resistance in some cases. For this reason, research of innovative anti-cancer agents is required. Certain plants have the capacity to prevent and/or fight against cancer due to many virtues that they contain. Compounds with biological activities are characterized by high performance analytical techniques such as liquid and gas chromatography tandem mass spectrometry [6]. *Myrmecodia pendans*, also named the ant nest plant and *sarang semut* in local language, is a South-East Asian native plant that has been traditionally and safely used in Indonesia to treat diverse diseases. Since 1950s, people of Papua were preparing the plant as a porridge mixture or boiled drink for its ability to boost the immune system. *M. pendans* displays an important antitumour activity as an implicit anticancer agent [7]. Therefore, it can inhibit various types of human cancers including colon, liver, prostate, blood, breast, brain, cervical, lung and skin [8]. Furthermore, it is a remedy for associated systemic diseases, like infectious disease, tuberculosis, leukaemia, kidney, heart and prostate diseases, besides different allergies, haemorrhoid, rheumatism and migraine [9]. The active substances reveal the effectiveness of the plant; it consists of flavonoids, tannins, polyphenols that insure the antioxidant function, as well as glycosides [10]. These active compounds of polyphenols are antimicrobial and antidiabetic, in addition to their anticancer properties. *M. pendans* is potent for surmounting cancer cells. Several cells, along with cancer cells, produce IL-8 which is a chemokine family oncoprotein. In fact, IL-8 levels tend to increase in progressive cancer. Cytokine protein IL-8 has an important function in the inflammatory process [11]. Indeed, in tumours and metastases, IL-8 promotes T cell infiltration, tumourigenesis, angiogenesis and chemoresistance [12].

In our study, the antiproliferative activity of *M. pendans* extracts in colon cancer cell was tested. We also analysed the effects of these *M. pendans* extracts on the secretion of IL-8 in the colon cells cancer.

### Materials and Methods

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) assay (Vybrant Cell proliferation assay), cell culture media and reagent, phosphate-buffered saline (PBS) and dimethyl sulfoxide (DMSO) were purchase from Invitrogen. Ethanol (99.8%) and distilled water were purchased from Sigma-Aldrich. HCT116 cell was kindly provided by Prof. Dumitrescu (The University of Agricultural Sciences and Veterinary Medicines of Timișoara, Romania). IL8 Human Elisa Kit was obtained from Boster Biological Technology (Pleasanton CA, USA, Catalogue ELISA Kit #EK0413). This study uses raw materials of *Myrmecodya pendans* obtained from the Papua region. The extraction was carried out from dry plant material in ethanol and water (10 g plant material *per* 100 mL solvent). The

solvents were evaporated and stock solutions were prepared in DMSO 1000 µg/mL for each solvent. From the stock solutions, the final test concentrations (150, 300, 450 and 600 µg/mL) were performed by dilution in culture media.

The cytotoxic potential evaluation of *M. pendans* extracts was performed using the colorimetric MTT assay.  $2 \times 10^4$  cells/mL ( $2 \times 10^3$  cells/well) were seeded in 100 µL media. HCT116 cells line were grown in Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEMF12) media (with or without test substances), at 37°C and 5% CO<sub>2</sub>. HCT116 cells were exposed to various concentrations of ethanol and aqueous extracts of *M. pendans* (150, 300, 450 and 600 µg/mL). After 24, 48 and 72 hours, 25 mL of MTT (5 mg/mL PBS) were added in the wells. After 4 hours of incubation at 37°C, the culture specific media was discarded and 100 mL of DMSO was added. Optical density at 540 nm was analysed (Tecan microplate reader). The test was performed in triplicate.

IL-8 levels were analysed using our own standard curve (optical density *vs.* concentration) in the case of 8 standard concentrations (0 - 15.6 - 31.2 - 62.5 - 125 - 250 - 500 - 1000 pg/mL). Elisa method (test range 15.6 - 1000.0 pg/mL) was performed to determine IL-8 level in the cell culture supernatant. The optical density was measured at 450 nm.

### Results and Discussion

*Myrmecodia pendans* originate from the eastern part of Indonesia. Some studies have emphasized that this plant may be useful in anticancer therapy. However, the extracts therapeutic effects and the active biological compounds activity of *M. pendans* are known in a lower manner. *M. pendans* extracts chemical analyses indicate that these plants contain flavonoid, tannin, as well as tocopherols, phenols and various minerals [10]. Also, some of the flavonoids mechanisms of action are known, such as the carcinogen inactivation, the antiproliferation, the cell cycle inhibition, the apoptosis induction, and the angiogenesis inhibition. A significant decrease in Burkitt lymphoma cell number was observed after the treatment with ethyl-acetate extract of *M. pendans*. There was a significant relationship between the concentrations of the extract, the period of incubation and the number of cells that can survive after being treated with the ethyl-acetate extract [13]. We attempted to evaluate the antiproliferative potential of the *M. pendans* ethanolic and aqueous extracts on HCT116 cancer cells. The MTT test results showed a moderate decrease in the cell multiplication rate. Thus, the cell proliferation inhibition was dose and time dependent for the ethanolic extracts at 24 and 72 hours incubation period. IC<sub>50</sub> (minimum inhibitory concentration) was 566.087 µg/mL for 72 h incubation time (Figure 1).

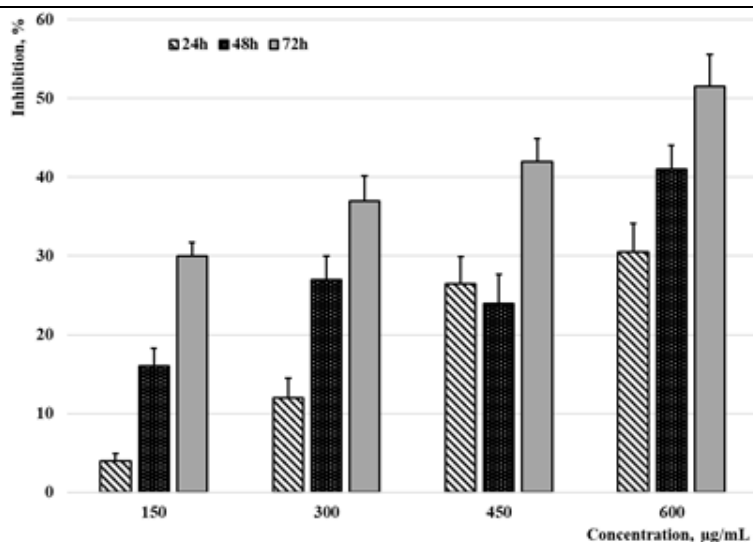


Figure 1.

Suppression of cell growth (MTT assay) – ethanolic extracts

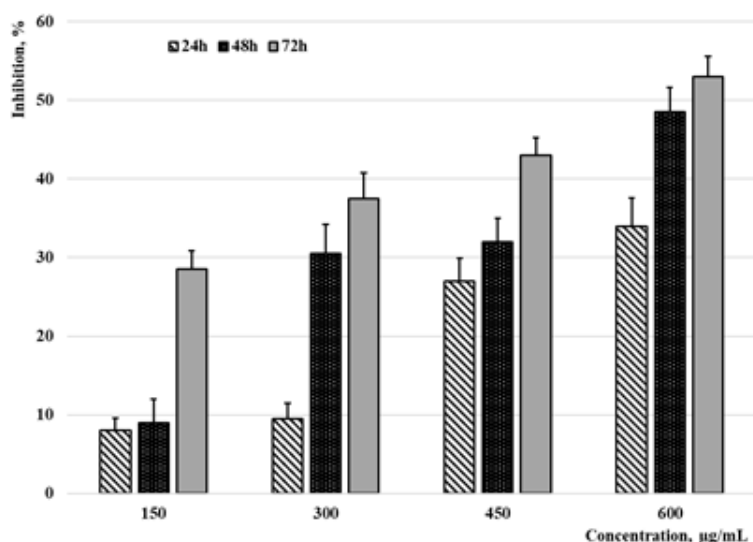


Figure 2.

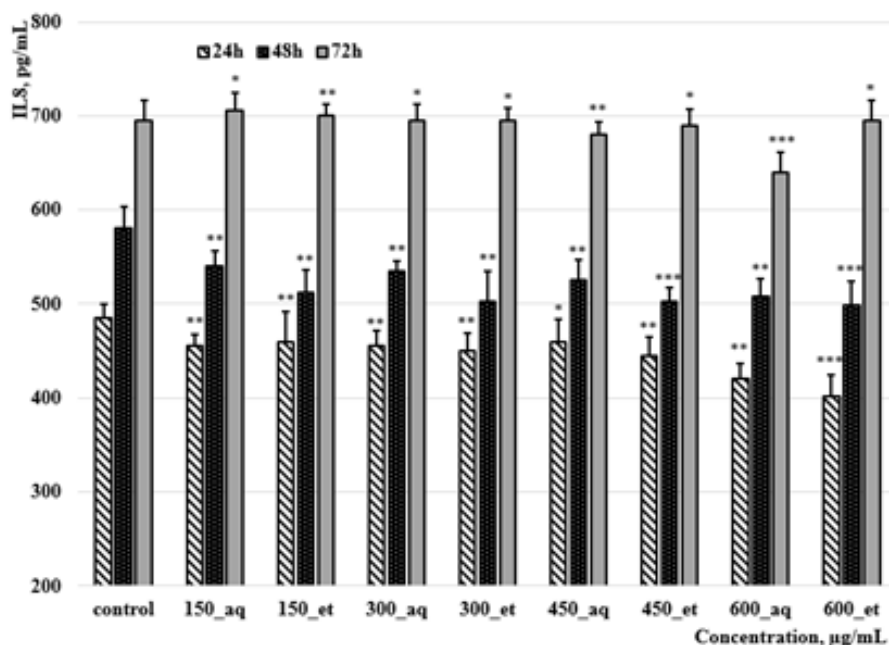
Suppression of cell growth (MTT assay) – aqueous extracts

IC<sub>50</sub> was not reached for the cells incubated with the ethanolic extracts at 24 and 48 hours. At 24, 48 and 72 hours incubation with aqueous extracts, the inhibition of HCT116 cell proliferation was dose and time-dependent (Figure 2); the IC<sub>50</sub> was 587.95 µg/mL, respectively 512 µg/mL at 48 and 72 h incubation. Although there are just a few studies in the literature describing the antitumoural potential of *M. pendans*, some research groups have already reported promising results. Thus, Achmad *et al.* have shown that in squamous cell carcinoma of tongue, the flavonoids obtained by extraction with ethyl-acetate possess antitumour activity on molecular signalling pathways including Akt and NFκB (nuclear transcription factor) [13]. Their cytotoxicity test results reported different values for IC<sub>50</sub> depending on the properties of the extraction solvent: ethyl-acetate, ethanol, hexane, and water, respectively 452.059, 938.003, 2691.535 and

12302.690 µg/mL. Another study reported a cell growth inhibition (Burkitt's lymphoma cancer cell) based on the concentration: the growth inhibition started from the lowest concentration of 15.625 µg/mL. Also, the incubation time (24, 48 and 72 hours) evidenced a direct proportionality with the cell growth inhibition rate, namely, the longer the incubation period is, the higher cell growth inhibition is [14]. A study realized by Soeksmanto *et al.* regarding the effects of *M. pendans* extracts on HeLa and MCM-B2 cell lines, reported that polar extracts, obtained in water, had a higher anticancer activity compared to non-polar extracts (extracts obtained in ethyl-acetate and n-butanol) [15]. N-hexane fraction of *M. pendans* extracts presented antitumour activity in colon cancer cells lines Caco2 and HCT116. However, the value of IC<sub>50</sub> differs depending on the colon cancer type, being higher for the HCT116 cells compared to the one for Caco2 cells

[16]. The decrease of cell proliferation, highlighted in the present study, can be explained by activating apoptosis pathways, fact suggested by some studies. In HSC-3 cells (human squamous carcinoma cells), *M. pendans* induces apoptosis by pro-apoptotic (Bax) and anti-apoptotic BCL-2 markers action [17]. *M. pendans* determines the apoptosis induction by both the extrinsic and intrinsic pathways, causing at the same time the induction of cyclin-dependent kinase p27Kip1 and cyclin E overexpression [18]. On normal fibroblasts cells, the alcoholic and aqueous extract

of *M. pendans* has no cytotoxic effect [19], which suggests the biocompatibility with healthy cells. Although, we have demonstrated the anti-proliferative effect of *M. pendans* aqueous and ethanolic extracts, the action on HCT116 cells did not caused significant changes in IL-8 synthesis. Despite the concentration or the incubation period with the extracts, IL-8 levels in the cell supernatant from the culture, were not significantly modified compared to the control (Figure 3). Interleukin 8 is a peptide in the cytokine family secreted by various cell types that exhibits a pro-inflammatory effect [8, 20].



**Figure 3.**

Concentrations of IL8 (pg/mL); aq = aqueous and et = ethanolic extracts

\*p < 0.05, \*\*p < 0.01 and \*\*\*p < 0.001 vs. control

The effects of the biological compounds from *M. pendans* on angiogenesis are currently under investigation; on the other hand, it is well-known that literature [21-22] describes IL-8 as a pro-angiogenic factor that facilitates the development of angiogenesis in a few cancers. Although several studies have demonstrated their therapeutic potential in different types of cancer, the mechanisms of action on cytokines and other molecules involved in angiogenesis are not yet elucidated.

### Conclusions

*M. pendans*, a plant from the *Rubiaceae* family, is often used by the Papuans as a medicinal plant that can treat tumours, gout, diarrhoea, and fever. Its extracts exert antiproliferative effects on neoplastic colonic HCT116, the cytotoxic effects being dependent on the extraction solvent properties, concentration and incubation period. In contrast, the doses studied did not significantly influence IL-8 secretion.

### Conflict of interest

The authors declare no conflict of interest.

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