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Antioxidant and cytotoxic potential of potentized preparation of *Cordyceps sinensis* in vitro in cancer cell lines

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Abstract

Background: The parasitic fungus *Cordyceps sinensis* (*Ophiocordyceps sinensis*) has been discovered in lepidopteran larvae, having known antitumor effects. Testing potentized *Cordyceps sinensis* in cancer cells could help broaden Homoeopathy cancer therapeutics.

Objectives: The objectives of the study were to evaluate the antioxidant and cytotoxicity activity of potentized preparation of *Cordyceps sinensis* in carcinoma cell-lines.

Methods: In this in vitro study, antioxidant activity was analyzed by the 2,2-diphenyl-1-picryl-hydrazyl-hydrate assay and the dilution with more antioxidant potential was further analyzed for cytotoxicity, using 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide assay on various carcinoma cell lines: Breast cancer cell line (MCF-7), liver cancer cell line (HePG2), lung cancer cell line (A-549), and prostate cancer cell line (PC3). All experiments were carried out in triplicates. Data were analyzed by one-way analysis of variance and the means were compared by Duncan's new multiple range test.

Results: A higher antioxidant potential 377.40 $\mu\text{L}/\text{mL}$ was seen in *Cordyceps sinensis* 30C, which was further analyzed for cytotoxicity in cell lines (MCF-7), (HePG2), (A-549), and (PC3) which it inhibited at concentrations $596.21 \pm 3.32 \mu\text{L}/\text{mL}$, $438.10 \pm 2.39 \mu\text{L}/\text{mL}$, $555.40 \pm 3.08 \mu\text{L}/\text{mL}$, and $656.42 \pm 2.68 \mu\text{L}/\text{mL}$, respectively. When comparing *Cordyceps sinensis* 30C to other cell lines, its cytotoxic activity against HEPG2 is particularly potent.

Conclusion: This research demonstrates the usefulness of potentized preparation of *Cordyceps sinensis*, it will take additional studies comparing it to currently used drugs to determine whether or not it is significantly more efficient.

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Antioxidant and cytotoxic potential of potentized preparation of *Cordyceps sinensis in vitro* in cancer cell lines

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Abstract

Background: The parasitic fungus *Cordyceps sinensis* (*Ophiocordyceps sinensis*) has been discovered in lepidopteran larvae, having known antitumor effects. Testing potentized *Cordyceps sinensis* in cancer cells could help broaden Homoeopathy cancer therapeutics. **Objectives:** The objectives of the study were to evaluate the antioxidant and cytotoxicity activity of potentized preparation of *Cordyceps sinensis* in carcinoma cell-lines. **Methods:** In this *in vitro* study, antioxidant activity was analyzed by the 2,2-diphenyl-1-picryl-hydrazyl-hydrate assay and the dilution with more antioxidant potential was further analyzed for cytotoxicity, using 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide assay on various carcinoma cell lines: Breast cancer cell line (MCF-7), liver cancer cell line (HePG2), lung cancer cell line (A-549), and prostate cancer cell line (PC3). All experiments were carried out in triplicates. Data were analyzed by one-way analysis of variance and the means were compared by Duncan's new multiple range test. **Results:** A higher antioxidant potential 377.40 $\mu\text{L/mL}$ was seen in *Cordyceps sinensis* 30C, which was further analyzed for cytotoxicity in cell lines (MCF-7), (HePG2), (A-549), and (PC3) which it inhibited at concentrations $596.21 \pm 3.32 \mu\text{L/mL}$, $438.10 \pm 2.39 \mu\text{L/mL}$, $555.40 \pm 3.08 \mu\text{L/mL}$, and $656.42 \pm 2.68 \mu\text{L/mL}$, respectively. When comparing *Cordyceps sinensis* 30C to other cell lines, its cytotoxic activity against HEPG2 is particularly potent. **Conclusion:** This research demonstrates the usefulness of potentized preparation of *Cordyceps sinensis*, it will take additional studies comparing it to currently used drugs to determine whether or not it is significantly more efficient.

Keywords: Anticancer drug, *Cordyceps sinensis*, *in vitro* study, *Ophiocordyceps sinensis*, Homoeopathy.

INTRODUCTION

Cancer is a complex set of disease symptoms that advances progressively with a general loss of growth control.^[1] According to GLOBOCAN, there were an estimated 19.3 million new cancer cases and nearly 10 million cancer deaths worldwide in 2020. In 2040, there are projected to be 28.4 million cancer cases worldwide, a 47% increase from 2020. With an estimated 2.3 million new cases, female breast cancer has exceeded lung cancer as the most frequently diagnosed cancer, followed by lung, colorectal, prostate, and stomach cancers. With an estimated 1.8 million deaths, lung cancer remains the leading cause of cancer-related mortality, followed by colorectal, liver, stomach, and female breast cancers.^[2] This disease is tissue-based, and the tissue variation greatly complicates diagnosis and treatment efficacy.^[3] In spite of advanced cancer treatments such as chemotherapy, immunotherapy, radiation therapy, surgery, and hormone therapy people use complementary and alternative therapies in view of their limited risk, natural origin, minimal consequences, and affordability.^[4]

According to recent international studies, 29–91% of cancer patients seek complementary and alternative medicine treatments.^[5] Patients with metastatic cancers would benefit from additional palliative care that would enhance their quality of life and allow efficient use of available medical resources.^[6] To support patients' lifestyle modifications, to manage both acute and chronic cancer-related symptoms, complementary therapies have been implemented at several oncological centers.^[7] Homoeopathy was reported as one of the most popular CIM therapies (40.4% of those used) in a 2015 European survey of 236 facilities providing integrative oncology services as part of the public health system.^[8] Several studies show that homoeopathy can help mitigate the toxic effects of oncology treatments, while

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also helping patients experience better health and overall well-being.^[9]

Ophiocordyceps sinensis (syn.-*Cordyceps sinensis*) is an entomopathogenic fungus that grows on insect larvae. More than 450 species linked to cordyceps are now found worldwide based on fungi or insect hosts.^[10] Harvesting begins in April and extends till August. This fungus only grows in the high-altitude regions of about 3800 m above sea level, where it can be found in alpine meadows on the Himalayan region.^[11] *Cordyceps* commonly grows on larvae (host) that typically reside 6 inches below the ground's surface. The fungus advances from the larval stage to maturity, consuming more than 90% of the insect host. The stroma reaches maturity, and it then bulges to form perihelia. A cordyceps weigh about 300–500 mg, which is typical.^[11] The reason to select this drug for this study is that its anti-cancer and anti-metastatic effects are established by targeting pathways such as Bcl-2/Bax,^[12] caspases,^[13] epidermal growth factor receptor,^[14] nuclear factor kappa B (NF-κB),^[15] phosphatidylinositol 3-Kinase/Protein Kinase B (PI3K–Akt),^[16] matrix metalloproteinase-2 (MMP-2)/MMP-9,^[17] Janus kinase/signal transducers and activators of transcription (JAK/STAT3)^[18] Mitogen-activated protein kinase (MAPK), and adenosine Monophosphate-activated protein kinase.^[19,20] Carcinoma cell lines of the prevailing cancers such as breast, liver, lung and prostate were taken for the present study.^[2] In a recent clinical trial conducted in partnership with Oxford University and NuCana, researchers discovered that NUC-7738, a novel drug for cancer treatment derived from *Ophiocordyceps sinensis*, was 40 times more effective in killing cancer cells than many of its parent compounds.^[21] *Cordyceps* is in extremely limited supply due to its unique environment, and as demand increases, the drug's value increases, making it an incredibly expensive drug. Homoeopathically prepared *Cordyceps sinensis* is used in the treatment of mental and physical exhaustion due to overwork; nerve weakness; chronic fatigue; mountain sickness, shortness of breath; and lack of strength and endurance. It increases athletic and general performance, especially in long-distance races. *Cordyceps sinensis* is used in the treatment of Alzheimer's disease, backache due to injury, fatigue, stress or simple aging. It is further used in the treatment of various conditions such as lumbar weakness, metrorrhagia, abnormal menstruation, increased appetite, tiredness, chronic fatigue syndrome, debility, immune weakness, anaemia, hypercholesterolemia, lymphoma, arrhythmias, weakness of heart, chronic obstructive hepatic diseases. It is therapeutically used for breathlessness, worse exertion, constricted bronchioles, asthma, persistent cough, mountain sickness, emphysema, lung cancer and tuberculosis. *Cordyceps sinensis* is also used for sexual hypofunction, sexual frigidity, infertility, loss of sexual drive, impotence and seminal emissions with aching of loins and knees. It is used in the preventing liver and spleen atrophy. It also antidotes opium and narcotic withdrawal symptoms.^[22]

The present study was done to investigate the antioxidant and anti-cancer potential of potentised *Cordyceps sinensis* using *in vitro* methods, in all the above-mentioned cell lines.

MATERIALS AND METHODS

Materials

Cordyceps sinensis 6C and *Cordyceps sinensis* 30C were procured from Helios Homoeopathy Limited, Tunbridge Wells, The United Kingdom. Phosphate Buffered Saline (PBS), 3-(4,5-dimethyl thiazol-2-yl)-5-diphenyl tetrazolium bromide (MTT), Dulbecco's Modified Eagle's medium (DMEM), fetal Bovine serum (FBS), and Trypsin were obtained from Sigma Aldrich Co, St Louis, USA. Ethylenediaminetetraacetic acid (EDTA), dimethyl sulfoxide (DMSO) Propanol from E. Merck Ltd., Mumbai, India, and glucose and antibiotics from Hi-Media Laboratories Ltd., Mumbai.

Cell lines

Human breast cancer cell line (MCF-7), human prostate cancer cell line (PC-3), lung cancer cell line (A-549), and liver cancer cell line (HEPG-2) were procured from National Centre for Cell Sciences Pune, India.

Culture medium

Cells were grown in stock in DMEM. The medium was supplemented with 10% inactivated FBS, penicillin (100 IU/mL), streptomycin (100 µg/mL), and amphotericin B (5 µg/mL) in a humidified atmosphere of 5% CO₂ at 37°C until confluent. The cells were dissociated with TPVG solution (0.02% EDTA, 0.2% trypsin, and 0.05% glucose in PBS). Experiments were carried out in 96 microtiter plates and the stock cultures were grown in 25 cm² culture flasks (Tarsons India Pvt. Ltd., Kolkata, India).

Methods

Evaluation of the antioxidant property in drug samples

To test the 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH radical scavenging activity), the Molyneux method^[23] was employed. In a test tube, 1.0 mL of 100.0 µM DPPH solution in methanol was mixed with an equal volume of the test samples of different-concentration solution in methanol and were incubated in the dark for 30 min. The color change was documented by measuring the light absorbance at 514 nm with a spectrophotometer. Instead of a test sample, only 1 mL of methanol was added to the control tube.

The DPPH radical scavenging activity of the sample was calculated as follows:

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of extract}}{\text{Absorbance of control}} \times 100$$

The IC₅₀^[24] value was determined using GraphPad Prism 5.0 by using linear regression,

$$y=mx+c$$

Where,

y = The dependent variable (i.e., what you measure as the signal)

x = The independent variable (i.e., what you control, such as, dose, and concentration)

m = The slope of the fitted line

c = The intercept of the dependent axis.

MTT assay

MTT assay (Denizot and Lang)^[25] is based on the ability of viable cells with active mitochondria to produce succinate dehydrogenase enzyme which cleave the tetrazolium rings of MTT, where the optical density obtained is proportional to the number of healthy viable cells.

Five samples for each drug were prepared for each cell line. The drug sample which had the highest antioxidant was selected and investigated. For cytotoxicity studies, each measured test drug sample was separately dissolved in distilled DMSO and the volume was made up with DMEM supplemented with 2% inactivated FBS to obtain a stock solution of 1 ml/ml concentration and sterilized by filtration. Serially 2-fold dilutions were prepared from this for carrying out cytotoxic studies.

Using a medium containing 10% FBS, the monolayer cell culture was trypsinized, and the cell count was increased to 1.0×10^5 cells/mL. A total of 0.1 mL of the diluted cell suspension (roughly 10,000 cells) was added to each well of the 96-well microtiter plate. When a partial monolayer had formed after 24 h, the supernatant was removed, the monolayer was washed once with medium, and then test drug concentrations ranging from 50 to 1000 $\mu\text{L}/\text{mL}$ were added on top of the partial monolayer in microtiter plates. The plates were subsequently incubated for 3 days at 37°C in a 5% CO_2 atmosphere, during which a microscopic examination was conducted and observations were recorded every 24 h. After 72 h, the drug solutions in the wells were discarded and 50 μL of MTT in PBS was added to each well. The plates were gently shaken and heated to 37°C in a 5% CO_2 atmosphere for 3 h. To dissolve the formazan that had formed, 100 μL of propanol was added after the supernatant was drained from the plates. A microplate reader calibrated to measure at 540 nm was used to measure absorbance. The following formula was used to determine the percentage growth inhibition, and the dose-response curves for each cell line were used to determine the concentration of test drug required to inhibit cell growth by 50% (CTC50).

% Growth inhibition =

$$100 - \frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \times 100$$

RESULTS

Antioxidant activity

The homoeopathic drugs, *Cordyceps sinensis* 6C and *Cordyceps sinensis* 30C, were analyzed using the DPPH

assay for scavenging activity with 90% v/v of ethanol as control. *Cordyceps sinensis* 6C at concentrations of 50 $\mu\text{L}/\text{mL}$, 250 $\mu\text{L}/\text{mL}$, 500 $\mu\text{L}/\text{mL}$, 750 $\mu\text{L}/\text{mL}$, and 1000 $\mu\text{L}/\text{mL}$ showed percentage inhibition of 33.77, 42.76, 48.9, 53.95, and 58.77, respectively. *Cordyceps sinensis* 30C at concentrations 50 $\mu\text{L}/\text{mL}$, 250 $\mu\text{L}/\text{mL}$, 500 $\mu\text{L}/\text{mL}$, 750 $\mu\text{L}/\text{mL}$, and 1000 $\mu\text{L}/\text{mL}$ showed percentage inhibition of 43.42, 47.37, 50.22, 59.65, and 64.47, respectively [Table 1].

A standard curve was plotted with the percentage of radical scavenging activity of ethanol, *Cordyceps sinensis* 6C and *Cordyceps sinensis* 30C in Figure 1. The IC50 value was calculated from the slope of the figure using the “y = mx + c” formula [Table 2]. The values of IC50 of ethanol, *Cordyceps sinensis* 6C, and *Cordyceps sinensis* 30C are 865.24 $\mu\text{L}/\text{mL}$, 603.83 $\mu\text{L}/\text{mL}$, 377.40 $\mu\text{L}/\text{mL}$, respectively, illustrated in [Figure 2]. The IC50 values obtained were significant ($P < 0.01$) for free radicals. IC50 for *Cordyceps sinensis* 30C was 377.40 $\mu\text{L}/\text{mL}$ and *Cordyceps sinensis* 6C was 603.83 $\mu\text{L}/\text{mL}$, thus *Cordyceps sinensis* 30C showed inhibitory activity at a much lower concentration. Since *Cordyceps sinensis* 30C had higher scavenging activity it was taken to the next stage of analyses for its cytotoxicity in different cancer cell lines.

Cytotoxic activity

Table 1: Mean value of % of inhibition of ethanol, *Cordyceps sinensis* 6C and *Cordyceps sinensis* 30C in different concentrations by DPPH assay

S. No.	Concentration ($\mu\text{L}/\text{mL}$)	Ethanol	<i>Cordyceps sinensis</i> 6C	<i>Cordyceps sinensis</i> 30C
		% of inhibition	% of inhibition	% of inhibition
1	50	21.27	33.77	43.42
2	250	26.31	42.76	47.37
3	500	35.74	48.9	50.22
4	750	46.71	53.95	59.65
5	1000	54.82	58.77	64.47

DPPH: 2,2-diphenyl-1-picryl-hydrazyl-hydrate

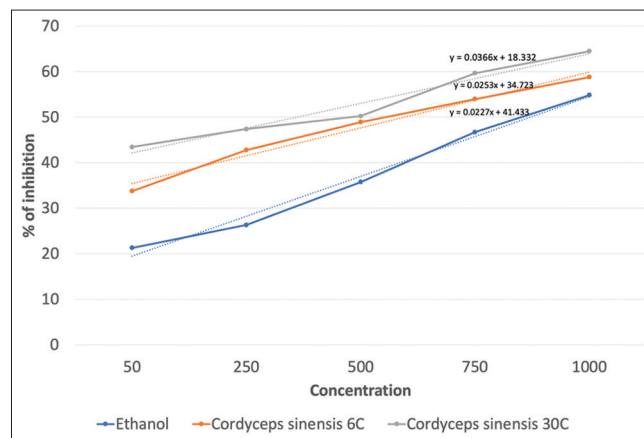


Figure 1: Comparison of mean value of % of inhibition of ethanol, *Cordyceps sinensis* 6C and *Cordyceps sinensis* 30C

Cordyceps sinensis 30C was tested using MTT assay with different concentrations, that is, 50–1000 µL/mL on MCF-7, HEPG2, and A-549 and PC3. Control tubes were kept which

Table 2: IC50 values of ethanol, *Cordyceps sinensis* 6C and *Cordyceps sinensis* 30C by using linear regression

IC50 Formula	Ethanol	<i>Cordyceps sinensis</i> 6C	<i>Cordyceps sinensis</i> 30C
$y=mx+c$	$y=0.0366x+18.33$	$y=0.0253x+34.72$	$y=0.0227x+41.43$
$x=(y-c)/m$	$x=(50-18.33)/0.03$	$x=(50-34.72)/0.02$	$x=(50-41.43)/0.02$
where, $y=50$			
IC50	865.24 µL/mL	603.83 µL/mL	377.40 µL/mL

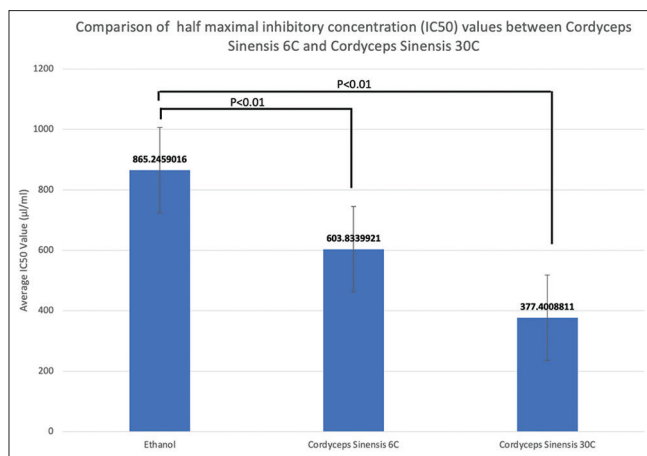


Figure 2: All experiments were carried out in triplicates. Data obtained were analyzed by one-way analysis of variance and means were compared by Duncan’s New Multiple Range test (SPSS 21.0 version). Representation of the results of DPPH assay, where ethanol has 865.24 µL/mL, *Cordyceps sinensis* 6C has 603.83 µL/mL and *Cordyceps sinensis* 30C has 377.40 µL/mL

consisted of saline and tumor cells without drug intervention [Figure 3]. *Cordyceps sinensis* 30C inhibited MCF7 cell line at a concentration of 596.21 ± 3.32 µL/mL, inhibited HEPG2 cell line at a concentration of 438.10 ± 2.39 µL/mL, inhibited A-549 cell line at a concentration of 555.40 ± 3.08 µL/mL, and inhibited PC3 cell line at a concentration of 656.42 ± 2.68 µL/mL [Figure 4].

DISCUSSION

Numerous previous studies on *Cordyceps* have demonstrated its wide-ranging medicinal properties, including those that are antimicrobial, fungicidal, larvicidal, inflammatory, diabetic, antioxidant, antitumor, pro-sexual, apoptotic, immunomodulatory, and anti-HIV.^[26] Some of the studies demonstrate that it inhibits M1 macrophage polarization mediated by activation of the NF-κB pathway, inhibiting inflammatory cytokines like MMP-9 can reduce tumor growth and metastasis.^[27] It can suppress the HEPG2 cell line of liver cancer cells by caspases triggered apoptosis, ergosterol increased lysosomal membrane permeability, by suppressing activated hepatic stellate cells.^[28] *Cordyceps sinensis* and cisplatin treatment significantly reduced cell viability, which subsequently caused a higher number of dead cells. Cordycepin suppressed MAPK and Rb/E2F1 signaling, cell cycle proteins, and fibroblast growth factor (FGF) receptor expression.^[29,30] It stimulated Fas, DR5, and caspase-8.^[31,32] Cordycepin’s anti-tumor properties resulted in a significant decline of cell viability in a dose-dependent and time-dependent manner. It was salient to understand the potential of potentized homeopathic *Cordyceps sinensis*. Hence, this pre-clinical study was done to evaluate the basic properties such as antioxidant and cytotoxicity of homeopathic *Cordyceps sinensis*, to test its efficacy to be an anticancer drug.

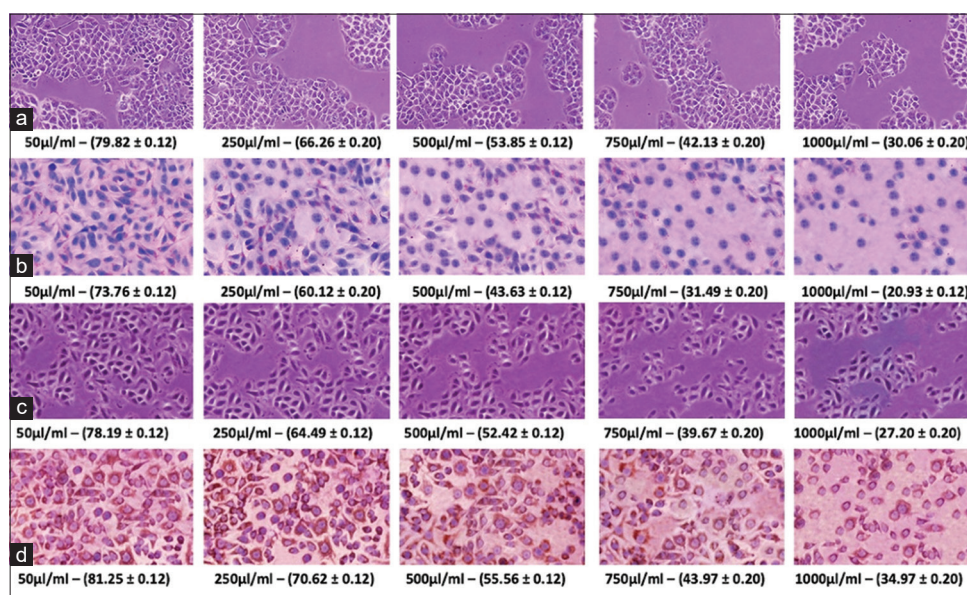


Figure 3: Representative photomicrographs of the cellular morphology and cell viability of (a) breast cancer cell line, (b) liver cancer cell line, (c) lung cancer cell line, (d) prostate cancer cell line at different concentrations

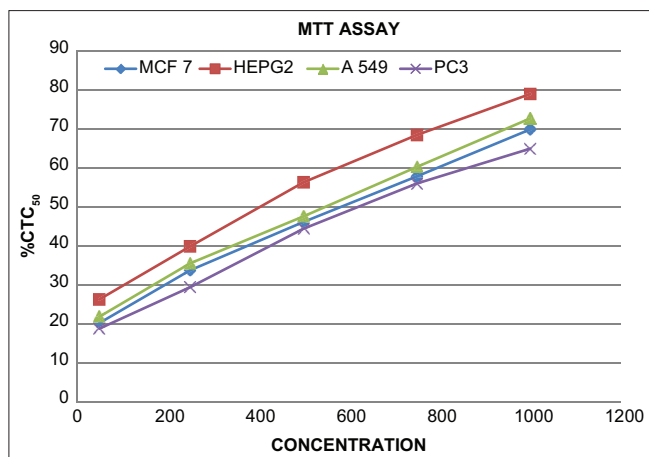


Figure 4: The mean value of drug concentration needed to inhibit breast cancer cell line is $596.21 \pm 3.32 \mu\text{L/mL}$, liver cancer cell line is $438.10 \pm 2.39 \mu\text{L/mL}$, lung cancer cell line is $555.40 \pm 3.08 \mu\text{L/mL}$, and prostate cancer cell line is $656.42 \pm 2.68 \mu\text{L/mL}$

In this study, it was found that *Cordyceps sinensis* 30C had higher antioxidant potential as compared to *Cordyceps sinensis* 6C when evaluated under the DPPH assay. *Cordyceps sinensis* 30C underwent MTT assay on different cancer cell lines, that is, human lung carcinoma (A549), hepatocellular (HEPG2), breast (MCF7), and prostate (P3). *Cordyceps sinensis* 6C was not further taken to test cytotoxicity because without enough antioxidant potential it can cause cell damage and oxidative stress which results in organoleptic damage comparing to *Cordyceps sinensis* 30C. According to the findings, *Cordyceps sinensis* 30C showed a significant cytotoxic activity in human lung carcinoma (A549), hepatocellular (HEPG2), breast (MCF7), and prostate (P3) cancer cell lines.

CONCLUSION

In this study, potentized preparation of *Cordyceps sinensis* has shown significant effects in all cell lines. Further studies must be done in comparison with prevailing drugs to know its effectiveness. Since significant anticancer properties are exhibited by the cytotoxic activity of *Cordyceps sinensis* 30C against HEPG2, compared to other cell lines, *in silico* molecular docking studies should be done to know the molecular affinity between HEPG2 and *Cordyceps sinensis* 30C, to understand the deeper aspects of its cytotoxic properties. Additional *in vivo* studies to assess the toxicological risk will help allay the safety concerns about the drug.

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Nil.

Conflicts of interest

None declared.

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Potentiel antioxydant et cytotoxique de la préparation potentialisée d'*Ophiocordyceps sinensis* in-vitro dans la lignée cellulaire du cancer du sein (MCF-7), la lignée cellulaire du cancer du foie (HePG2), la lignée cellulaire du cancer du pulmonaire (A-549), la lignée cellulaire du cancer de la prostate (PC3)

Contexte: Le champignon parasite *Ophiocordyceps sinensis* a été découvert dans des larves de lépidoptères, ayant des effets antitumoraux connus. Tester le *Cordyceps sinensis* dynamisé dans des cellules cancéreuses pourrait aider à élargir les thérapies anticancéreuses homéopathiques. **Objectif:** Évaluer l'activité antioxydante et cytotoxique d'une préparation dynamisée de *Cordyceps sinensis* dans des lignées cellulaires de carcinome. **Méthodes:** Dans cette étude in-vitro, l'activité antioxydante a été analysée par le test DPPH (2,2-diphényl-1-picryl-hydrazyl-hydrate) et la dilution avec plus de potentiel antioxydant a été analysée plus avant pour la cytotoxicité, en utilisant le MTT (3-(bromure de 4,5-diméthyl-thiazol-2-yl)-2,5-diphényltétrazolium) sur différentes lignées cellulaires de carcinome : lignée cellulaire de cancer du sein (MCF-7), lignée cellulaire de cancer du foie (HePG2), lignée cellulaire de cancer du pulmonaire (A-549), lignée cellulaire du cancer de la prostate (PC3). Toutes les expériences ont été réalisées en triple. Les données ont été analysées au moyen d'une analyse unidirectionnelle de la variance (ANOVA) et les moyennes ont été comparées par le test Nouvelle Multiple Range de Duncan. **Résultat:** Un potentiel antioxydant plus élevé de 377,40 µl/ml a été observé dans le *Cordyceps sinensis* 30C, qui a ensuite été analysé pour la cytotoxicité dans les lignées cellulaires (MCF-7), (HePG2), (A-549), (PC3), ont été il a inhibé aux concentrations 596,21 ± 3,32 µl/ml, 438,10 ± 2,39 µl/ml, 555,40 ± 3,08 µl/ml, 656,42 ± 2,68 µl/ml, respectivement. Lorsque l'on compare *Cordyceps sinensis* 30C à d'autres lignées cellulaires, son activité cytotoxique contre les lignées cellulaires du cancer du foie (HEPG2) est particulièrement puissante. **Conclusion:** Cette recherche démontre l'utilité de la préparation potentialisée de *Cordyceps sinensis*, il faudra des études complémentaires la comparant aux médicaments actuellement utilisés pour déterminer si oui ou non elle est significativement plus efficace.

Antioxidatives und zytotoxisches Potenzial der potenzierten Zubereitung von *Ophiocordyceps sinensis* in-vitro in der Brustkrebszelllinie (MCF-7), der Leberkrebszelllinie (HePG2), der Lungenkrebszelllinie (A-549), der Prostatakrebszelllinie (PC3)

Hintergrund: Der parasitäre Pilz *Ophiocordyceps sinensis* wurde in Lepidoptera-Larven entdeckt und ist für seine antitumorale Wirkung bekannt. Die Prüfung von potenziertem *Cordyceps sinensis* in Krebszellen könnte dazu beitragen, die Krebstherapien der Homöopathie zu erweitern. **Zielsetzung:** Bewertung der antioxidativen und zytotoxischen Aktivität einer potenzierten Zubereitung von *Cordyceps sinensis* in Karzinomzelllinien. **Methoden:** In dieser In-vitro-Studie wurde die antioxidative Aktivität mit Hilfe des DPPH-Tests (2,2-Diphenyl-1-picryl-hydrazyl-hydrat) analysiert, und die Verdünnung mit dem höheren antioxidativen Potenzial wurde mit Hilfe des MTT-Tests (3-(4,5-Dimethyl-thiazol-2-yl)-2,5-diphenyltetrazoliumbromid) an verschiedenen Karzinomzelllinien auf Zytotoxizität untersucht: Brustkrebszelllinie (MCF-7), Leberkrebszelllinie (HePG2), Lungenkrebszelllinie (A-549), Prostatakrebszelllinie (PC3). Alle Experimente wurden in dreifacher Ausführung durchgeführt. Die Daten wurden mittels einseitiger Varianzanalyse (ANOVA) analysiert, und die Mittelwerte wurden mittels Duncan's New Multiple Range Test verglichen. **Ergebnisse:** *Cordyceps sinensis* 30C wies ein höheres antioxidatives Potenzial von 377,40 µl/ml auf, das in den Zelllinien (MCF-7), (HePG2), (A-549), (PC3) auf Zytotoxizität untersucht wurde, wobei die Konzentrationen 596. 21 ± 3,32 µl/ml, 438,10 ± 2,39 µl/ml, 555,40 ± 3,08 µl/ml, 656,42 ± 2,68 µl/ml. Vergleicht man *Cordyceps sinensis* 30C mit anderen Zelllinien, so ist seine zytotoxische Aktivität gegen Leberkrebs-Zelllinien (HEPG2) besonders stark. **Schlussfolgerung:** Diese Forschung zeigt die Nützlichkeit der potenzierten Zubereitung von *Cordyceps sinensis*. Es bedarf weiterer Studien, in denen sie mit den derzeit verwendeten Arzneimitteln verglichen wird, um festzustellen, ob sie wesentlich wirksamer ist oder nicht.

स्तन कैंसर की कोशिका रेखा (MCF-7), यकृत कैंसर की कोशिका रेखा (HePG2), फेफड़े के कैंसर की कोशिका रेखा (A-549), और प्रोस्टेट कैंसर की कोशिका रेखा (PC3) में ओफियोकोर्डिसेप्स सिनेनसिस इन-विट्रो की पोटेन्टाइज़्ड पदार्थ की एंटीऑक्सीडेंट और साइटोटॉक्सिक क्षमता

पृष्ठभूमि: लेपिडोप्टेरा लार्वा में परजीवी कवक ओफियोकोर्डिसेप्स सिनेनसिस की खोज की गई है, जिसके ट्यूमर रोधी प्रभाव ज्ञात हैं। कैंसर कोशिकाओं में पोटेन्टाइज़्ड कॉर्डिसेप्स सिनेनसिस का परीक्षण होम्योपैथी कैंसर चिकित्सा विज्ञान को विस्तृत करने में मदद कर सकता है। **उद्देश्य:** कार्सिनोमा कोशिका रेखाओं में पोटेन्टाइज़्ड कॉर्डिसेप्स सिनेनसिस की एंटीऑक्सीडेंट तथा साइटोटॉक्सिक क्षमता का आंकलन करना। **विधि:** इस इन-विट्रो अध्ययन में, एंटीऑक्सीडेंट क्षमता का विश्लेषण DPPH (2,2-डाईफिनाइल-1-पिक्रिल-हाईड्राजिल-हाइड्रेट) जांच के माध्यम से किया गया और विभिन्न कार्सिनोमा कोशिका रेखाओं: स्तन कैंसर कोशिका रेखा (MCF-7), यकृत कैंसर कोशिका रेखा (HePG2), फेफड़े के कैंसर की कोशिका रेखा (A-549), और प्रोस्टेट कैंसर की कोशिका रेखा (PC3) पर MTT (3-(4,5-डाईमिथाइल-थायाजोल-2-वाइएल)-2,5- डाईफिनाइल टेट्राजोलियम ब्रोमाइड) जांच का उपयोग करते हुए साइटोटॉक्सिक के लिए अधिक एंटीऑक्सीडेंट क्षमता वाले विलयन की जांच की गई। सभी प्रयोगों को तीन बार किया गया। डाटा का विश्लेषण विचरण के एक तरफ़ा विश्लेषण (अनोवा) से किया गया और औसत (मीन) की परस्पर तुलना डंकन के न्यू मल्टीपल रेंज परीक्षण द्वारा की गई थी। **परिणाम:** कॉर्डिसेप्स सिनेनसिस 30C में 377.40 µl/ml की उच्च एंटीऑक्सीडेंट क्षमता देखी गई, जिसका साइटोटॉक्सिक विश्लेषण (MCF-7), (HePG2), (A-549), (PC3)

कोशिका रेखाओं में किया गया जिनमें इनकी सांद्रता क्रमशः $596.21 \pm 3.32 \mu\text{l/ml}$, $438.10 \pm 2.39 \mu\text{l/ml}$, $555.40 \pm 3.08 \mu\text{l/ml}$, $656.42 \pm 2.68 \mu\text{l/ml}$ पाई गई। से कॉर्डिसेप्स सिनेनसिस 30C की साइटोटॉक्सिटी क्षमता अन्य कोशिका रेखाओं की तुलना में यकृत कैंसर की कोशिका रेखाओं (HEPG2) के विरुद्ध विशेषकर प्रबल पाई गई। **निष्कर्ष:** इस शोध से पोटेन्टाइज़्ड कॉर्डिसेप्स सिनेनसिस की उपयोगिता प्रदर्शित होती है, कि वर्तमान में उपयोग की जाने वाली दवाओं की तुलना में यह काफी अधिक कुशल है या नहीं, यह निर्धारित करने के लिए, अतिरिक्त अध्ययन कि आवश्यकता पड़ेगी।

Potencial antioxidante y citotóxico de la preparación potenciada de *Ophiocordyceps sinensis en-vitro* en línea celular de cáncer de mama (MCF-7), línea celular de cáncer de hígado (HePG2), línea celular de cáncer de pulmón (A-549), línea celular de cáncer de próstata (PC3)

Fondo: El hongo parásito *Ophiocordyceps sinensis* ha sido descubierto en larvas de lepidópteros, teniendo efectos antitumorales conocidos. La prueba de *Cordyceps sinensis* potenciado en células cancerosas podría ayudar a ampliar la terapéutica del cáncer homeopático. **Objetivo:** Evaluar la actividad antioxidante y citotóxica de la preparación potenciada de *Cordyceps sinensis* en líneas celulares de carcinoma. **Métodos:** En este estudio en-vitro, se analizó la actividad antioxidante mediante el ensayo DPPH (2,2-difenil-1-picril-hidrazilo-hidrato) y la dilución con más potencial antioxidante se analizó más a fondo para determinar la citotoxicidad, utilizando el ensayo MTT (3-(4,5-dimetil-tiazol-2-il)-2,5-difeniltetrazolio bromuro) en varias líneas celulares de carcinoma: línea celular de cáncer de mama (MCF-7), línea celular de cáncer de hígado (HePG2), línea celular de cáncer de pulmón (A-549), línea celular de cáncer de próstata (PC3). Todos los experimentos se llevaron a cabo por triplicado. Los datos se analizaron mediante análisis unidireccional de varianza (ANOVA) y las medias se compararon mediante la prueba de Nueva Rango Múltiple de Duncan. **Resultado:** Se observó un mayor potencial antioxidante de $377,40 \mu\text{l/ml}$ en *Cordyceps sinensis* 30C, que se analizó más a fondo para determinar la citotoxicidad en líneas celulares (MCF-7), (HePG2), (A-549), (PC3), donde se inhibió a concentraciones $596,21 \pm 3,32 \mu\text{l/ml}$, $438,10 \pm 2,39 \mu\text{l/ml}$, $555,40 \pm 3,08 \mu\text{l/ml}$, $656,42 \pm 2,68 \mu\text{l/ml}$ respectivamente. Al comparar *Cordyceps sinensis* 30C con otras líneas celulares, su actividad citotóxica contra las líneas celulares de cáncer de hígado (HEPG2) es particularmente potente. **Conclusión:** Esta investigación demuestra la utilidad de la preparación potenciada de *Cordyceps sinensis*, se necesitarán estudios adicionales que la comparen con los medicamentos utilizados actualmente para determinar si es o no significativamente más eficiente.

增强型冬虫夏草制剂体外对乳腺癌细胞株 (MCF-7)、肝癌细胞株 (HePG2)、肺癌细胞株 (A-549)、前列腺癌细胞株 (PC3) 的抗氧化和细胞毒作用。

背景: 在鳞翅目幼虫中发现了寄生真菌冬虫夏草, 具有已知的抗肿瘤作用。在癌症细胞中测试增强的冬虫夏草可能有助于拓宽癌症同源病治疗方法。 **目的:** 评价冬虫夏草制剂对癌细胞系和的抗氧化和细胞毒性作用。 **方法:** 在本体外研究中, 通过DPPH (2,2-二苯基-1-苦基-胍基-水合物) 法分析抗氧化活性, 并通过MTT (3-(4,5-二甲基噻唑-2-基)-2,5-二苯基四唑溴化铵) 法对各种癌细胞系 (乳腺癌细胞系 (MCF-7)、癌症细胞系 (HePG2)、肺癌细胞系 (A-549)、前列腺癌细胞系 (PC3))。所有实验分三次进行。通过单因素方差分析 (ANOVA) 对数据进行分析, 并通过邓肯新多范围检验对平均值进行比较。 **结果:** 冬虫夏草30C具有较高的抗氧化能力 $377.40 \mu\text{l/ml}$, 进一步分析了其在细胞系 (MCF-7)、(HePG2)、(A-549)、(PC3) 中的细胞毒性。当将冬虫夏草30C与其他细胞系进行比较时, 其对癌症细胞系 (HEPG2) 的细胞毒活性尤为显著。 **结论:** 本研究证明了潜在的冬虫夏草制剂的有效性, 需要进一步的研究将其与目前使用的药物进行比较, 以确定其是否更有效。