

Achillea gonocephala Extract Loaded into Nanochitosan: In Vitro Cytotoxic and Antioxidant Activity

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ABSTRACT

Introduction: The present study aimed to prepare *Achillea gonocephala* chloroform extract and evaluate antioxidant and cytotoxic effects. Then, the nanoparticles (NPs) were synthesized on the most efficient extracts and the biological activities of the free forms of the extracts were compared with the NPs forms.

Methods: Antioxidant capacities of 14 extracts (A-N) prepared using column chromatography were determined by FRAP, DPPH and CUPRAC methods. Again, the cytotoxic activities of all fractions were evaluated on MCF-7 and HT-29 cell lines using the XTT cell viability assay. Chitosan-tripolyphosphate (TPP) NPs of the extracts with the most active biological properties were formed using the ionic gelation method.

Results: The particle size of the NPs synthesized from the two most efficient extracts was found to be between 274.12 and 296.25 nm. The extract-encapsulation and loading-efficiency of the most active NPs were found 77.6±0.04% and 7.76±0.01% for the F extract and 10.2±0.02 and 1.39±0.07% for the H extract, respectively.

Conclusions: While antioxidant activity results of chitosan NPs were close to unencapsulated extracts, cytotoxic activity results were found to be better than unencapsulated extracts. Based on these results, it is thought that it would be more effective to use the encapsulated forms of *A. gonocephala* instead of the raw extract of the plant in the treatment.

Keywords: Nanoparticles, Cytotoxicity, Antioxidant, Chitosan, Encapsulation efficiency

1. INTRODUCTION

Cancer is known as the formation of undesired tissues by uncontrolled division and proliferation of cells in the organism. There are many factors in the epidemiology of the disease, such as lifestyle and unhealthy diet, stress, radiation, and hereditary factors (1). Recently, natural compounds of plant origin, called phytochemicals, have attracted the attention of researchers in the field of pharmacy and medicine worldwide due to their highly important bioactive properties. Nowadays, studies on phytochemicals are performed in the treatment of various diseases (2). *Achillea* species have proven to have analgesic and anti-inflammatory, erythrocyte, and leukocyte preservative, antispasmodic (3) antioxidant and antimicrobial (4) anthelmintic, antiallergic, cholagogue, antibacterial (5) antiulcer, hepatoprotective and anxiolytic (6) effects. In addition, sesquiterpene lactones, alkaloids (senecio), esters (pyrethrins); Saponosides and coumarins have also been identified in this family. Due to the compounds, they contain, most of the *Achillea* plants are used in pharmacy, food industry and other industrial

areas due to their latex (7). *A.gonocephala* Boiss. & Balansa (*A. gonocephala*) is an endemic plant and when looking at the literature, there are only two studies on this species and these studies are also related to essential oil analysis. In a study, the essential oil of *A. gonocephala* was analyzed by GC/MS. Camphor and 1,8-cineole were found to be the main ingredients (8). In another study, the essential oil of this plant was analysed and high amount endo-borneol, eucalyptol and camphor were found (9).

With some new nanotechnologies such as encapsulation, preservation of active substances, reduction of activity loss and increasing bioavailability have been proven in recent years (10). The difficulties in converting the phytochemicals contained in plants into drugs are known due to reasons such as low solubility, stability, and bioavailability. Thanks to the nanostructures formed because of adding plant extracts to the nanoparticles (NPs), it has been revealed that the stability and especially the biological activities of the phytochemicals

in the plant are increased (11). Therefore, the application of nanotechnology to plant extracts can be considered a good strategy for herbal medicines, as it provides many features such as improving pharmacological activity and especially protection from toxicity. Chitosan, which is the most used and non-toxic polymer among the coating materials used in encapsulation, shows a good encapsulation feature. Chitosan is biodegradable, biocompatible, antibacterial, and non-toxic, and besides these properties, it is an interesting material with its abundance and cheapness. For this reason, it is used in many areas such as medicine, food, pharmacy, cosmetics, textile, agriculture, and the removal of environmental pollutants from wastewater. Due to its unique properties, chitosan is widely used in drug delivery systems (12).

In this study, antioxidant, and cytotoxic activities of 14 fractions obtained using column chromatography from *A. goniocephala* chloroform extract, which was found to have a stronger effect than methanol extract in MCF-7 cell line, were evaluated. Then, NPs were prepared with two most active fractions by combining with chitosan. Thus, the biological activities of the pure forms of the extract/fractions with the biological activities of the NP forms were compared. No study has been found in the literature on the antioxidant and cytotoxic activity of the *A. goniocephala*. In addition, plant extract loaded NP synthesis and characterization study was carried out by us for the first time.

2. METHODS

2.1. Materials

The specimens of *A. goniocephala* were collected from field studies and determined by Prof. Dr Turan Arabacı. A voucher specimen (T.Arabacı 2957) was deposited at the herbarium of the Faculty of Pharmacy, İnönü University, Department of Pharmaceutical Botany for future reference. MCF-7 human breast cancer cells, and HT-29 human colorectal adenocarcinoma cells were obtained from the American Type Culture Collection (ATCC). Dulbecco's modified Eagle's medium (DMEM) (ATCC, USA), phosphate buffer saline (PBS), and fetal bovine serum (FBS) were purchased from PAA Ltd. (France). Chitosan (400 kDa, DD 87) were obtained from Fluka. L-glutamine-penicillin-streptomycin solution were purchased from Sigma-Aldrich. XTT reagent (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) was purchased from Roche Diagnostic.

2.2. Extraction procedures

The aerial parts of the plant were dried in the shade (25°C) and ground to a fine powder in a mechanic grinder (Renas, RBT1250). The powdered samples were extracted with organic solvents (methanol, and chloroform) using the maceration method until colourless. The organic phase was evaporated to dryness under decreased pressure. All extracts were maintained at 4°C for future analysis. The cytotoxic activities of chloroform ($IC_{50} = 0.019$ mg/mL) and methanol

($IC_{50} = 20.391$ mg/mL) extracts were assessed on the MCF-7 cell line, and it was observed that the chloroform extract was more potent against MCF-7 than methanol extract. In this study, column chromatography was performed to separate the possible effective compounds contained in the effective chloroform extract according to its polarity. The chloroform sub-extract (7 g) was inserted to a silica gel column and eluted with toluene, petroleum ether and methanol mixture of increasing polarity of petroleum ether (100%, 1500 mL), petroleum ether /chloroform (75:25, 1500 mL), petroleum ether /chloroform (1:1, 1500 mL), chloroform (100%, 1500 mL), chloroform/methanol (75:25, 1500 mL), chloroform/methanol (1:1, 1500 mL), chloroform/methanol (25:75, 1500 mL), and methanol (100%, 1000 mL) to obtain fourteen fractions.

2.3. Antioxidant assays

Plant samples were tested with Ferric Reducing Antioxidant Power (FRAP) assay, where an increase in plant extracts' absorbance shows increase in the reducing power of the extracts (13). The FRAP results were expressed as mM Fe^{2+} /mg extract. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity of the extracts was evaluated the experimental protocol found in the literature (14). Results are expressed as IC_{50} values (mg/mL). In Cupric Reducing Antioxidant Capacity (CUPRAC) assay, 60 μ L each of copper (II) solution, neocuproin solution and ammonium acetate buffer (1 M) were mixed. 10 μ L ethanol and 60 μ L of extract were added and shake the solution. The solutions were kept in room conditions with their mouth closed for 60 minutes. At the end of this period, absorbance values at 450 nm were measured against the reference solution that does not contain a sample (15). CUPRAC values were given as mM TroloxE/mg extract.

2.4. Cell culture conditions

Cell culture studies were performed using modified method of (16). Cytotoxicity of the extracts was measured by the XTT assay, using HT-29 and MCF – 7 cells. Cells were cultured in medium glucose DMEM with 10% FBS, 1% L-glutamine, 100 IU/mL penicillin and 10 mg/mL streptomycin in 25 cm^2 polystyrene flasks. The cells were maintained at 37°C within 5% CO_2 humidified atmosphere and were passaged when they had reached 80-90% confluence. Cells were seeded at 10×10^3 cells each well in 96-well plates containing DMEM (100 μ L) containing 10% FBS and incubated overnight. The 14 extracts were dissolved in 20 μ L dimethyl sulfoxide (DMSO) and extracts with concentration 500 μ g/mL were suspended with DMEM medium and extracts at the concentration of 50 μ g/mL per well were put in the 96-well plates. In addition, the equal amount of DMSO were inserted in the positive control. Incubation of the cells was continued for 24 h. Then, the medium was removed, and wells were washed with PBS. To determine cell viability, 100 μ L of transparent (colourless) DMEM and 50 μ L of XTT labelling solution were added to wells and the cells were incubated for 4 h. Microplate (ELISA)

reader was used to measure the absorbance of XTT-formazan at 450 nm against the control. Cell viability of extracts were calculated compared to control (100% of viability). After the XTT results were evaluated, NPs of the two extracts showing the highest cytotoxicity in MCF-7 and HT-29 cells were prepared. NPs containing different amounts plant extract were applied to the cells separately, and the differences between their cytotoxic effects were evaluated by calculating the IC_{50} values. To calculate the IC_{50} value, samples and NPs containing 12.5, 25, 50, and 100 μg amount of plant extracts were treated with the cells.

2.5. Preparation of chitosan NPs containing chloroform extract

NPs were prepared using ionic gelation method as stated (17). Briefly, medium molecular weight chitosan solutions at a concentration (0.25% w/v) were prepared using glacial acetic acid (0.05% v/v) as a solvent. The chitosan dissolution process was performed via a magnetic stirrer. TPP solution (0.25% w/v) including extract dropped into chitosan solution under predetermined stirring condition. After the stirring period, NPs were centrifugated at 10.000xg for 30 minutes. The supernatant was removed, and particles were washed with double distilled water. This process was repeated threefold. NPs were maintained at +4 °C after lyophilisation.

2.6. Characterization of NPs

Zeta potential and particle size: Zeta sizer were used to measure of zeta potential and size of NPs. The samples were suspended in PBS (pH 7.4) and measured. All measurements were performed in triplicate.

Scanning electron microscope (SEM): Sample of NP was inserted in metal grids with double-sided adhesive tape, coated with a gold layer under 0.1 torr at room temperature. The morphological properties of NPs were evaluated using SEM (Carl Zeis-Evo 40, Germany) (18).

2.7. Measurement of EE and LC

Ultraviolet visible spectrophotometer was used to measure the encapsulation efficiency (EE %) and loading capacity (LC %) of the extract in NPs. A standard calibration curve of the extract was established at 340 nm. The amount of extract in the supernatant was calculated from the line equation obtained from this curve. The following equations were used to determine the encapsulation efficiency and loading capacity of the particles.

$$EE (\%) = \frac{m_0 - m_s}{m_0} \times 100$$

$$LC (\%) = \frac{m_0 - m_s}{w_{np}} \times 100$$

where, m_0 is the initial mass of natural extracts and m_s mass of natural extracts in the supernatant and w_{np} = total weight of the naturally obtained extract of NPs (19). All measurements were performed in triplicate and were reported as mean \pm SD ($n = 3$).

Total phenolic contents in the extracts and NPs were determined by Folin-Ciocalteu colorimetric method. The percentage of the encapsulated extract into chitosan NP was also determined by using a total phenolic assay according to the following equation (20).

$$EE (\%) = \frac{\text{Actual phenolic of the extract entrapped in NPs}}{\text{total phenolic of initial extract}} \times 100$$

2.8. Statistical analysis

All experiments were performed in triplicate. All data from the study were given as the mean \pm SD and analysed using Graphpad Prism 5. Statistical differences between the study groups were analysed using a two-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Mean values were considered statistically significant if $p < 0.05$.

3. RESULTS

3.1. Antioxidant activity

The DPPH radical scavenging activities of the extracts were determined using DPPH method. The antioxidant activities of the extracts and ascorbic acid used as a standard were evaluated by comparing their IC_{50} values. H extract had stronger DPPH radical scavenging potential than the other extracts. The Copper (II) ion-reducing antioxidant capacity of the extracts was evaluated by the CUPRAC method. When CUPRAC results were compared, it was determined that the J, F G and H extracts exhibited the strongest copper (II) ion-reducing antioxidant capacity. The iron (III) ion reducing power of plants is very important in evaluating their antioxidant potential. The iron reduction power is based on the reduction of the herbal extract Fe^{3+} to Fe^{2+} and measured spectrometrically at 593 nm. In this method, high absorbance indicates high iron reduction potential. In this study, the antioxidant power of iron (III) ion reduction of different extracts obtained from the plant was examined comparatively. It was determined that G extract and F extract had stronger iron (III) ion reducing power than other extracts. All the extracts were found to have lower FRAP values than BHT compound (Table 1).

Table 1. Antioxidant capacity results of the fractions, references and nanocapsules

Extracts/ compound	DPPH (IC ₅₀ : mg/mL)	CUPRAC (mM trolox /mg extract)	FRAP (mM FeSO ₄ /mg extract)
A	0.611±0.218 ^a	0.240±0.045 ^a	9.791±0.304 ^a
B	0.561±0.077 ^b	0.044±0.015 ^b	9.750±0.181 ^{b,a}
C	1.032±0.224 ^c	0.306±0.008 ^c	10.355±0.160 ^c
D	1.178±0.280 ^d	0.116±0.038 ^d	11.585±0.716 ^d
E	1.536±0.356 ^e	0.212±0.016 ^e	10.496±0.070 ^{e,c}
F	0.895±0.062^f	2.582±0.158^f	11.746±0.209^f
G	0.809±0.101 ^g	2.006±0.158 ^g	13.540±0.384^g
H	0.264±0.037^h	1.111±0.038^h	10.879±0.152^h
I	1.349±0.635 ⁱ	1.050±0.010 ⁱ	10.980±0.244 ^{u,h}
J	1.266±0.278 ^j	3.155±0.172^j	11.565±0.218 ^j
K	0.890±0.289 ^k	0.780±0.047 ^k	10.395±0.185 ^{k,c}
L	1.543±0.210 ^l	0.702±0.060 ^l	10.416±0.060 ^l
M	2.012±0.688 ^m	0.664±0.095 ^m	10.436±0.152 ^{m,l,e}
N	2.499±0.423 ⁿ	0.412±0.017 ⁿ	10.154±0.175 ⁿ
H capsule	1.054±0.163 ^o	0.155±0.032 ^o	8.780±0.740 ^o
F capsule	1.426±0.060 ^p	0.759±0.040 ^{p,k}	8.659±0.083 ^{p,o}
Ascorbic acid	0.0028±0.000 ^r		
BHT		4.462±0.074 ^r	21.706±0.726 ^r

All results were expressed as mean±SD by three independent experiments; Means with different superscripts (a-r) are significantly different, $p < 0.05$.

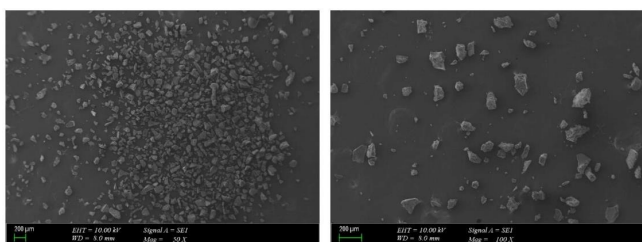
3.2. Characterization of chitosan NPs

Particle size, zeta potential, polydispersity index (PDI) of NPs were evaluated and results shown in Table 2. The size of the NPs obtained between 274.12±2.7 nm and 296.25±3.2 nm. The zeta potential values of NPs were between 2.3±0.04 mV and 3.0±0.03 mV. PDI values were between 0.298±0.03 and 0.342±0.04. These results showed that the samples were homogeneous feature without any aggregate. The morphological properties of the NPs were shown in Figure 1.

Table 2. Zeta potential, particle size, and PDI values of NPs loading with extract

Formulations (Chitosan – TPP NPs)	Zeta potential (mV)±SD	Size (nm)±SD	PDI±SD
*NP1	2.3±0.04	274.12±2.7	0.298±0.03
*NP2	2.7±0.02	280.15±3.6	0.313±0.03
*NP3	3.0±0.03	296.25±3.2	0.342±0.04

* NP1, NP2, and NP3 containing chitosan (MW: 400 kD), TPP and A. *goniocephala* chloroform extract. Nanoparticles: NPs.

**Figure 1.** SEM images of chitosan nanoparticles.

3.3. Percentage of Encapsulation efficiency from A. *goniocephala* extracts-chitosan NP

Encapsulation efficiency percentage suggests how much A. *goniocephala* F and H extracts coated in chitosan NP. The percentage of the encapsulated extract into chitosan NP was determined and the results were given in Table 3. As shown in Table 3, the percentage was found 77.6% and 10.2% for F and H fractions, respectively. This showed that as much as 77.6 or 10.2 % of the fractions are successfully encapsulated by chitosan NPs. Also loading capacity (LC) in NPs was found as 7.76 and 1.39 % for F and H fractions, respectively. According to the total phenolic method, the encapsulation efficiency percentages were 64.37% for the F extract and 14.84% for the H extract.

Table 3. Encapsulation efficiency and loading capacity of the NPs

Parameter	F extract	H extract
Linear equation	$y = 0.2808x + 0.02339$	$y = 0.4667x - 0.002409$
Slope±SD	0.2808±0.02	0.4667±0.01
Intercept±SD	0.0234±0.0011	0.002409±0.0007
r	0.9921	0.9925
EE %	77.60±0.04	10.20±0.02
LC %	7.76±0.01	1.39±0.07
TPC*	0.0174±0.0005	0.0566±0.0010
NP	0.0112±0.0003	0.0084±0.0003
EE % (TPC)	64.37±0.06	14.84±0.11

* TPC: Total phenolic contents (mg gallic acid equivalent / mg extract), Nanoparticles: NPs.

3.4. Cytotoxicity results of extracts, fractions, and NPs

Cancer is the second most common cause of death globally, despite the use of many treatment methods (21). Crucial side effects caused by conventional drugs used for chemotherapy affect patients negatively. Current studies have focused on natural herbal remedies, which are more advantageous than conventional cancer drugs and have anticancer effects (22,23). The cytotoxic activities of chloroform extracts from the plant on MCF-7 and HT-29 cell lines were examined, and results were shown in Figure 2 and 3. Results indicated that F and H extracts possessed more cytotoxic activity on both MCF-7 and HT-29 cell lines compared to other chloroform extracts. On MCF-7 cell line, the cell viability ranged between 51.691±0.566% and 85.019±0.637% (Figure 2). According to the results F and N samples of the extracts of plant showed the highest and lowest cytotoxic effect on MCF-7 cell line, respectively. In addition, H samples showed high cytotoxic activity on MCF-7 cell line (57.502±0.904%).

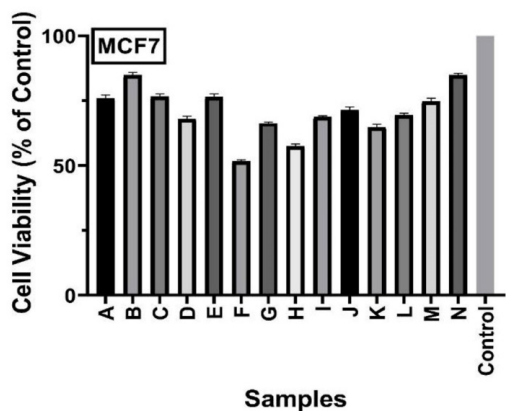


Figure 2. Cell viability results of chloroform extracts of the plant. Cells were treated with samples at a concentration 50 μ g/mL. Cell viability of the control group was determined as 100%.

On HT-29 cell line, the cell viability was between 49.368 \pm 0.569 % and 82.642 \pm 0.593 % (Figure 3). According to the results F and N samples of the plant extracts showed the highest and lowest cytotoxic effect on HT-29 cell line respectively. In addition, H samples showed high cytotoxic activity on HT-29 cell line (57.502 \pm 0.904%). In parallel with the cell viability results of MCF-7 cells, the highest cytotoxic effect was observed in F and H samples in HT-29 cells where all extracts were applied separately (F: 54.504 \pm 0.574% and H: 49.368 \pm 0.569%). Moreover, N samples showed the least cytotoxic activity (82.642 \pm 0.593%).

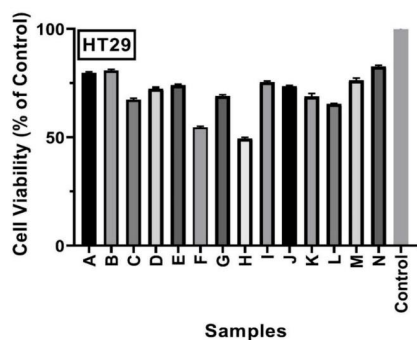


Figure 3. Cell viability results of chloroform extract of the plant. Cells were treated with samples at a concentration 50 μ g/mL.

When we evaluated the cytotoxic effects of the extracts on MCF-7 and HT-29 cells, it was observed that F and H chloroform extracts had stronger cytotoxic potential. Based on these results, NPs containing F and H extracts were prepared, and the extracts and NPs containing the extracts were treated with MCF-7 and HT-29 cells at different concentrations. These concentrations were determined to be I:12.5 μ g/mL, II: 25 μ g/mL, III: 50 μ g/mL, and IV: 100 μ g/mL. XTT cell viability test was performed in both cell lines at determined concentrations and IC₅₀ values were evaluated. When we evaluate the results in Figure 4, the NPs containing the same concentration of extract showed a higher cytotoxic effect in MCF-7 cancer cells compared to the samples containing

the extract. In cells treated with different concentrations (I, II, III, and IV) of F extract, the viability of MCF-7 cells was between 76.068 \pm 1.704% and 38.586 \pm 0.881%. In addition, cells treated with NPs including same concentrations (I, II, III, and IV) of F extract, the viability of MCF-7 cells was between 65.185 \pm 0.976% and 32.110 \pm 1.747%. In cells treated with different concentrations of H extract, the viability of MCF-7 cells was between 79.997 \pm 0.658% and 40.918 \pm 0.294%. Moreover, cells treated with NPs including same concentrations of H extract, the viability of MCF-7 cells was between 68.548 \pm 1.216% and 35.726 \pm 0.655%. According to the results of the XTT cell viability assay, it can be said that depending on the concentration of the extracts applied, it has a higher cytotoxic effect and anticancer effect in MCF-7 cells. IC₅₀ values of F extract and NP loading with F extract were calculated as 56.780 μ g and 40.860 μ g, respectively, on MCF-7 cells. In addition, IC₅₀ values of H extract and NP containing H extract were calculated as 61.630 μ g and 44.524 μ g, consecutively, on MCF-7 cells. FI NP (32.110 \pm 1.747%) and HI NP (35.726 \pm 0.655%) NP samples indicated the highest cytotoxic effect on MCF-7 cell line. NPs including extracts had lower IC₅₀ values than extracts on MCF7 – cell line (Figure 4).

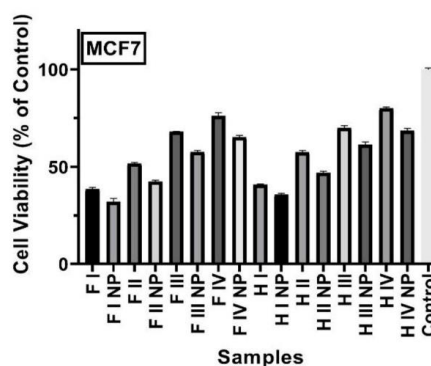


Figure 4. Cell viability results of F and H chloroform extract and NPs. MCF-7 cells were treated with samples at determined concentration (I:12.5 μ g/mL, II: 25 μ g/mL, III: 50 μ g/mL, and IV: 100 μ g/mL). Cell viability of the control group was determined as 100%.

According to the results of Figure 5, the NPs including the plant extract had a greater cytotoxic activity in HT-29 cancer cells compared to the samples containing the extract. In cells treated with different concentrations (I, II, III, and IV) of F extract, the cell viability of HT-29 cells was between 80.408 \pm 0.825% and 43.596 \pm 0.882%. In addition, cells treated with NPs containing same concentrations (I, II, III, and IV) of F extract, the cell viability of HT-29 cells was between 66.521 \pm 0.976% and 35.440 \pm 1.221%. In cells treated with predetermined concentrations of H extract, the cell viability of HT-29 cells was between 77.237 \pm 1.155% and 42.580 \pm 0.761%. Moreover, cells treated with NPs including same concentrations of H extract, the viability of HT-29 cells was between 69.552 \pm 0.891% and 31.560 \pm 0.642%. According to the results, IC₅₀ values of F extract and NP containing F extract were calculated as 64.460 μ g and 45.482 μ g, respectively, on HT-29 cells. Moreover, IC₅₀

values of H extract and NP loading with H extract were calculated as 51.280 μg and 40.860 μg , consecutively, on HT-29 cells. In addition, FI NP (35.440 \pm 1.222%) and HI NP (31.560 \pm 0.642%) NP samples showed the highest cytotoxic effect on HT-29 cell line (Figure 5).

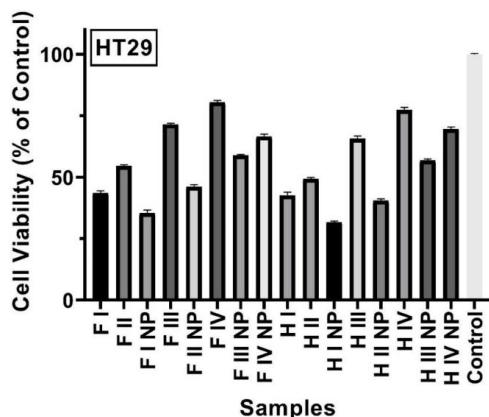


Figure 5. Cell viability results of F and H chloroform extract and nanoparticles. HT-29 cells were treated with samples at determined concentration (I: 12.5 $\mu\text{g}/\text{mL}$, II: 25 $\mu\text{g}/\text{mL}$, III: 50 $\mu\text{g}/\text{mL}$, and IV: 100 $\mu\text{g}/\text{mL}$). Cell viability of the control group was determined as 100%

4. DISCUSSION

Results showed that chitosan NPs including extracts had lower IC_{50} values than only extracts. In a study, it was proved that *C. casia* extract, which is widely used for antihypercholesterol in Indonesia, can be encapsulated with chitosan NPs and this NP has a cholesterol-lowering effect (19). In another study, chitosan NPs of anticancer effective elagic acid were created. It has been observed that the encapsulated elagic acid gives a lower IC_{50} value for oral cancer treatment compared to pure elagic acid (24). In a study, gelatin-based NPs of anticancer-effective, tannic acid, curcumin, theaflavin and epigallocatechin gallate polyphenols were prepared, and among these molecules, it was found that both free and nanocapsulated forms of epigallocatechin gallate were effective in breast cancer (25). In a study, the efficiency and release of the encapsulation potential of catechins was investigated and for this purpose, it was found that more stable results were obtained in in vitro and in vivo studies with chitosan NPs that they created by adding reducing substances to catechins (26). In a study, PGA-based NP was produced with the double emulsion technique of curcumin. They proved that the drug release rate of the NP was shorter without hydrolytic degradation in the presence of curcumin (27). In another study, NPs loaded with antioxidant hydroxytyrosol, and hydrocortisone were produced. As a result of the study, it was reported that this dual NP structure has an antioxidant and anti-inflammatory effect in the treatment of percutaneous atopic dermatitis (28). In a review study, studies in which nanoencapsulated polyphenols were produced to eliminate the negativities

such as weak bioavailability in the therapeutic applications of polyphenols were compiled. As a result of this review, they stated that polyphenols are more effective than their therapeutically pure forms, such as determining their release in nanoencapsulated form, extending the shelf life of active ingredients (29). There is another review in the literature based on the investigation of biopharmaceutical properties of extracts obtained from plants according to lipid and polymer-based NPs. As a result of this review, it was concluded that due to the controlled kinetic release of NPs produced using plant extracts, the effect of increasing the effect against microorganisms such as antioxidants and parasites is increased (30). They found that the IC_{50} value of green tea decreased after the nanoencapsulation created with chitosan, thus increasing the antioxidant effect (31). In another study, they found that a very small dose of *P. dactylifera* loaded chitosan NPs prevented the toxicity of doxorubicin by the development of antioxidant enzymes (32). Biosynthesized AgNPs were found to show a higher antioxidant and antibacterial activity compared to *Prosopis farcta* fruit extract alone (33). Similarly, in our study, chitosan NPs containing the most active extracts were prepared and characterization studies were carried out. In cell culture studies, the anti-proliferative and anticancer effects of the extracts embedded in chitosan, a carrier system, were evaluated. IC_{50} values calculated according to XTT cell viability results explain that NPs containing F and H chloroform extracts of *A. goniocephala* have desirable high anticancer activity.

Encapsulation efficiency is a measure of the ability of the carrier system used to embed the active substance. The encapsulation efficiency of the active substances in the formulations is crucial in terms of bioactivity and pharmacokinetic efficiency. Therefore, for the prepared formulations to show the desired effectiveness in practice, the encapsulation efficiency should be above a certain value. In our study, while the encapsulation efficiency of the F extract was 77.6%, this value was 10.2% in the H extract. When the encapsulation efficiency results were examined, it was observed that especially the F extract was successfully encapsulated by ionic gelation method.

5. CONCLUSION

According to the results of the XTT cytotoxicity and all the antioxidant assays study, among 14 extracts, F and H chloroform extracts of *A. goniocephala* showed better antioxidant and cytotoxic activity. While antioxidant activity results of chitosan NPs were close to unencapsulated extracts, cytotoxic activity results were found to be better than unencapsulated extracts. Based on these findings, it is anticipated that using the encapsulated forms of *A. goniocephala* rather than the raw extract of the plant in the treatment would be more successful.

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