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## Research Article

# Cytogenetic Toxicity of *Juniperus procera* Extract with Silver Nanoparticles Against Carcinoma Colon (Caco2) Cell Line *in vitro*

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### Abstract

**Background and Objective:** Medicinal plant species represent a large source of new compounds that help for the preparation of new drugs. In this research was evaluated the cytotoxic activity of leaves and fruits extract of *Juniperus procera*, commonly used in folk medicine in Saudi Arabia against Carcinoma colon (Caco2) cell lines. **Materials and Methods:** Caco2 cell lines were exposed to different concentrations of leaves and fruits extract of *J. procera* with silver nanoparticles (AgNPs). The MTT assay was used to determine the cytotoxic effect of all treatments. Morphology of Caco2 cell lines was monitored using an inverted microscope. Nuclei of Caco2 cell lines was counted using hemocytometer chamber. The DNA fragmentation of Caco2 cell lines was separated electrophoretically on a 2% agarose gel containing  $1 \mu\text{g mL}^{-1}$  ethidium bromide and visualized under ultraviolet transillumination. **Results:** Toxicity percentage of leaves and fruits extracts of *J. procera* against Caco2 cells was dose dependent, with the concentrations. Fruits extract was more effective ( $\text{IC}_{50}$   $8.80 \mu\text{g mL}^{-1}$ ) than leaves ( $\text{IC}_{50}$   $11.44 \mu\text{g mL}^{-1}$ ). Mixing  $\text{IC}_{50}$  of AgNPs (47.32 ppm) with  $\text{IC}_{50}$  of leaves or  $\text{IC}_{50}$  of fruits showed strong positive anti-cancer activity where the toxicity reached to 65.39 and 74.44%, respectively. Number of nuclei in treated cell decreasing with increasing extract concentration and not detected at high concentration of leaves ( $500 \mu\text{g mL}^{-1}$ ) and fruits extract ( $250$  and  $500 \mu\text{g mL}^{-1}$ ). The DNA of treated cells with  $\text{IC}_{50}$  of fruit and leaves extract remained intact as in the controls and DNA smearing was not detected but DNA fragmentation was clear with  $\text{IC}_{50}$  of AgNPs treatment. **Conclusion:** Present investigation concluded that the obtained  $\text{IC}_{50}$  fruits or leaves *J. procera* extract against Caco2 cells meaning that the extracts have potential anti-cancer properties.

**Key words:** *Juniperus procera*, cytotoxicity, silver nanoparticles, carcinoma colon cells, anti-cancer properties

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**Competing Interest:** The author has declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Cancer is a pathological condition characterized by excessive cell growth and deriving from loss of control over the cell cycle and/or decreased apoptosis<sup>1</sup>. Colon cancer is one of the most common types of cancers worldwide. While chemotherapy is one of the most widely used therapeutic strategies against colon cancer, it also has some limitations, such as normal cell toxicity and gradually increasing resistance in cancer cells. The discovery of new drugs for use in alternative strategies in cancer treatment is therefore highly desirable. Plants are regarded as very promising from this perspective, since they represent substantial sources of substances with various therapeutic uses. Most anti-cancer drugs are today produced from plants<sup>2,3</sup>.

All developing countries and Gulf countries, most of the population still depends on folk medicine to treat serious diseases including cancers and various types of inflammations. Nowadays, use of medicines from plant source increases significantly with conventional therapies. Hence, the plants are gaining more attention by the researchers to find out new and effective agents for different diseases<sup>4</sup>. Over 50% of drugs used in clinical trials for anti-cancer activity have been isolated from natural sources or are related to them. Hence, the search for natural products to be used in cancer therapy represents an area of great interest in which the plant kingdom has been the most important source, providing many anti-tumor agents with novel structures and unique mechanisms of action<sup>5</sup>. About 61% of new drugs developed between 1981 and 2002 were based on natural products and they have been very successful, especially in the areas of infectious disease and cancer<sup>6</sup>. The herbal medicines have a vital role in the prevention and treatment of cancer and they are also commonly accessible<sup>7</sup>. Natural extracts and biologically active compounds isolated from plant species used in traditional medicine could be resources for new drugs<sup>8-11</sup>.

*Junipers* are long lived trees which sometimes live up to 2000 years. It is belong to the Cupressaceae family. Economic importance of genus *Juniperus* was attributed to its various phytochemical constituents as coumarins and flavonoids<sup>12</sup>, phenylpropanoid<sup>13</sup> and essential oils<sup>14</sup>. *Junipers* species are known for their potential as a source for two important chemical products, the anti-cancer drug synthetic precursor, podophyllotoxin and essential oils<sup>15</sup>. The medicinal uses of *Juniperus* spp. are widespread in many countries such as Saudi Arabia, Bosnia, Lebanon and Turkey and according to folk medicine was used for treating skin and respiratory tract diseases<sup>16</sup>, urinary problems, rheumatism and gall bladder stones<sup>17</sup>. *Juniperus procera* is used in the

southern part of Saudi Arabia as a traditional remedy for tuberculosis and jaundice<sup>18</sup>. Three alpha-hinokiol (3) and 3 alpha-hydroxymannol (9) isolated from *Juniperus przewalskii*, exhibited effective anti-tumor activities to cervical carcinoma (HeLa) and human ovarian carcinoma (HO-8910) cell lines<sup>19</sup>. *Juniperus chinensis*, commonly known as Chinese juniper, is a native and widely used ornamental plant in East Asian countries. The *J. chinensis* and plants of the same genus exhibit many bio-activities, such as anti-microbial, anti-fungal, anti-viral, anti-insect, anti-fertility, vasorelaxing and anti-tumor activities<sup>20</sup>. Several anti-parasitic, nematicidal, anti-microbial, anti-mycobacterial and hepatoprotective compounds were isolated from bark, leaves and berries of *J. procera* including abieta-7, 13-diene, ferruginol, isocupressic acid, (+)-Z-communic acid, (+)-totarol, 4-epi-abietol and sugiol<sup>21</sup>.

Several studies indicated that deoxypodophyllotoxin isolated from *Juniperus communis* and from bark of *J. procera*<sup>22</sup> significantly induced cell apoptosis of breast cancer cells<sup>15</sup> and non-small cell lung cancer cells<sup>23</sup>. Previous studies reported anti-cancer activity of *J. phoenicea*<sup>24,25</sup>. Native Americans used *J. communis* berries as an appetite suppressant and in the treatment of diabetes<sup>26</sup>, antioxidant<sup>27</sup>, antimicrobial<sup>28</sup> activity. *Juniperus excelsa* berries extract and its fractions showed good and moderate levels of tumor inhibition<sup>29</sup>. All scientific reports reflect the unique properties silver nanoparticles (AgNPs) possess that find myriad applications such as antibacterial, antifungal and anti-cancer drugs, very good antioxidants, treatment of diabetes-related complications and wound healing activities<sup>30-34</sup>. The anti-tumor properties of AgNPs may be a cost-effective alternative in the treatment of cancer<sup>35</sup>. The purpose of this study was therefore to determine the cyto and genotoxic effect of *J. procera* leaves and fruits extract against colon cancer cell lines, one of the most common forms of cancer worldwide, with assessment the cytotoxicity of AgNPs combined with *J. procera* extracts.

## MATERIALS AND METHODS

**Location and total time duration of research work:** Location of research work in King Abdulaziz University, Jeddah, Saudi Arabia. Work planed begin at November, 2018 and continued to March, 2019.

**Collection and preparation of plant extracts:** The *J. procera* aerial parts (leaves and fruits) were collected in November, 2018 from Fifa mountains, Jizan Region, southwest Saudi

Arabia. The plant was identified according to Migahid<sup>36</sup> and Chaudhary<sup>37</sup>. The fresh leaves and fruits (100 g for each it) of *J. procera* were air dried at room temperature under shade and ground into powder using an electric grinder. About 10 g from dried powder of leaves and fruits were incubated with 50 mL ethanol with shaking overnight, the extract was filtrated using filter paper and then drayed and the dry weight measured as 1.25 and 0.82 g for leaves and fruits, respectively.

**Silver nanoparticles (AgNPs) used:** The AgNPs (chemically synthesized <100 nm) were obtained from Sigma-Aldrich.

**Cell line:** The Caco2 cells supplied by the America Type Culture Collection (ATCC, USA) were used (Organism, homo sapiens human, tissue, colon, cell type, epithelial, culture properties, adherent, disease, colorectal adenocarcinoma, ATCC, ATB-37).

**Cytotoxicity of plant extract (leaves and fruits) and AgNPs:**

Viable cells were measured by the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay according to MTT kit (R and D Systems) manufacturer's instructions. Caco2 cells were inoculated in 96 well tissue culture plates at  $1 \times 10^5$  cells mL<sup>-1</sup> (100  $\mu$ L well<sup>-1</sup>) and incubated at 37°C for 24 h to develop a complete monolayer sheet. Growth medium was decanted from 96 well micro titer plates after confluent sheet of cells were formed, cell monolayer was washed twice with wash media. Two-fold dilutions of tested sample were made in RPMI medium with 2% serum (maintenance medium). Each dilution (0.1 mL) was tested in different wells leaving 3 wells as control, receiving only maintenance medium. Plate was incubated at 37°C and examined. Cells were checked for any physical signs of toxicity, e.g., partial or complete loss of the monolayer, rounding, shrinkage or cell granulation. The MTT solution was prepared (5 mg mL<sup>-1</sup> in PBS) (BIO BASIC CANADA INC.,) then 20  $\mu$ L MTT solution were added to each well. Place on a shaking table, 150 rpm for 5 min, to thoroughly mix the MTT into the media. Incubate (37°C, 5% CO<sub>2</sub>) for 1-5 h to allow the MTT to be metabolized. Resuspend formazan (MTT metabolic product) in 200  $\mu$ L DMSO. Place on a shaking table, 150 rpm for 5 min, to thoroughly mix the formazan into the solvent. Read optical density at 560 nm and subtract background at 620 nm. Optical density should be directly correlated with cell quantity. The OD of formazan formed in control cells was taken as 100% of viability and the positively stained cells with MTT are expressed as the percentage (%) compared to

control. Log concentrations versus (%) cell viabilities were plotted with a logarithmic graph, which was then used to determine the IC<sub>50</sub> values<sup>38</sup>.

**Morphological analysis:** The morphology of cell was monitored using an inverted microscope. The Caco2 cells were checked for morphologic changes after 48 h exposure to range concentrations of plant extract or as compared to control and photographs were taken<sup>39</sup>.

**Combination assay of fruits and leaves extracts of**

***J. procera* with AgNPs:** Combined assay of IC<sub>50</sub> of fruits and leaves of *J. procera* with AgNPs (47.32 ppm) was evaluated to determine its toxicity on Caco2 cells using MTT assay as described previously<sup>40</sup>.

**Nuclei counting of treated Caco2 cells:** The treated cells incubated in a mixture of citric acid and crystal violet that causes cells to lyse and the released nuclei to stain purple. Allow micro carriers from a culture sample (1 mL) to settle to the bottom of a centrifuge tube. Then the clear supernatant was removed by aspiration, 1 mL of crystal violet reagent was added and incubate at 37 at least 1 h. Introduce a sample into the hemocytometer chamber to count the purple-stained nuclei for whole cells as following: Volume of cell solution (mL)  $\times$  Dilution factor in PBS blue (1:10)  $\times$  Mean number of stained cells  $\times 10^4$  (Conversion of 0.1 mm<sup>3</sup> to mL)<sup>40</sup>.

**DNA fragmentation:** The culture medium of treated cells by IC<sub>50</sub> of fruits (8.80  $\mu$ g mL<sup>-1</sup>) and leaves (11.44  $\mu$ g mL<sup>-1</sup>) of *J. procera* extract and AgNPs (47.32 ppm) was removed and centrifuged at 3000 rpm for 5 min to collect detached cells. Adherent cells were lysed with a hypotonic lysis buffer (10 mM Tris-HCl, pH 8.0) containing EDTA (10 mM) and Triton X-100 (0.5%) and then pooled with pellets made of detached cells. RNA was digested using RNase (0.1 mg mL<sup>-1</sup> at 37°C for 1 h) followed by proteinase K treatment for 2 h at 50°C. The DNA was extracted with a mixture of phenol, chloroform and isoamyl alcohol (25:24:1). The DNA was precipitated by adding an equal volume of isopropyl alcohol, stored overnight at 20°C and centrifuged at 12,000 rpm for 15 min at 4°C. The pellet was air-dried, resuspended in 20  $\mu$ L Tris acetate EDTA buffer supplemented with 2  $\mu$ L of sample buffer (0.25% bromphenol blue, 30% glyceric acid) and electrophoretically separated on a 2% agarose gel containing 1  $\mu$ g mL<sup>-1</sup> ethidium bromide and visualized under ultraviolet transillumination<sup>41</sup>.

**Statistical analysis:** The results are reported as mean±standard error S.E. of three independent replicates. Statistical analysis of data were carried out by computer using SPSS ver. 22.0 software.

## RESULTS

**Cytotoxicity of *J. procera* extract and AgNPs:** Leaves and fruits extracts of *J. procera* were tested against Caco2 colorectal carcinoma cells to determine their ability to inhibit cancer cell growth (Table 1). Toxicity (%) of leaves and fruits extracts of *J. procera* against Caco2 cells were dose dependent, with the concentrations. Toxicity (%) of leaves and fruits extracts of *J. procera* was similar up to 125 µg mL<sup>-1</sup>. However, fruits extract was more effective because its IC<sub>50</sub> (8.80 µg mL<sup>-1</sup>) was less than leaves (11.44 µg mL<sup>-1</sup>) extracts. The IC<sub>50</sub> of leaves and fruits extracts of *J. procera* indicated the strongest active against Caco2. Further evaluation of AgNPs outlined that lowest level of cytotoxic activity against Caco2 cells at concentration less than 62.5 µg mL<sup>-1</sup> with the IC<sub>50</sub> values of 47.32 µg mL<sup>-1</sup> but more than 62.5 µg mL<sup>-1</sup> concentration, it showed highest toxicity (Table 1).

**Combined effect of IC<sub>50</sub> of AgNPs with *J. procera* extract on Caco2 cell line:** The synergistic potential of AgNPs IC<sub>50</sub> together with the IC<sub>50</sub> of leaves and fruits extracts were tested against Caco2 cells (Table 2). Mixing AgNPs IC<sub>50</sub> (47.32 ppm) with leaves IC<sub>50</sub> (11.44 µg mL<sup>-1</sup>) or fruits IC<sub>50</sub> (8.80 µg mL<sup>-1</sup>) showed strong positive anti-cancer activity

where the toxicity reached to 65.397 and 74.44%, respectively. But unfortunately 1/10 IC<sub>50</sub> of leaves or fruits antagonize the activity of AgNPs against Caco2 cells where the toxicity was 47.46 and 40.64%, respectively. Nuclei not detected at high concentration of leaves (500 µg mL<sup>-1</sup>) and fruits (250 and 500 µg mL<sup>-1</sup>) extract. At high concentration (250 and 500 ppm) of AgNPs, nuclei was detected (Table 3).

**Effect of *J. procera* extract and AgNPs on morphological features of Caco2 cell line:** Morphological features of Caco2 cells treated with different concentration of leaves extract of *J. procera* were reported (Fig. 1) and compared with the untreated cells. At 250 and 500 µg mL<sup>-1</sup> concentrations of leaves extracts of *J. procera* the treated cells showed remarkable difference with the control. Also, morphological changes of Caco2 cells treated with fruits extract of *J. procera* were reported (Fig. 2), where destructuration of cells were clear at high concentration up to IC<sub>50</sub> (8.80 µg mL<sup>-1</sup>) of fruits extract. From the current results, the cells treated with fruits extract was more affected than treated with leaves extract, where the cells appeared less uniform with the loss of membrane integrity, rounding, shrinkage, however still intact at 31.25 and 62.50 µg mL<sup>-1</sup>.

On the other hand, Identifiable morphological features of apoptosis were observed in the treated cells with AgNPs (Fig. 3), morphological changes were clear in a concentration-dependent manner in case AgNPs treatment.

Table 1: Cytotoxicity of *J. procera* extract (leaves and fruits) and AgNPs against Caco2 cells

Concentration (µg mL <sup>-1</sup> )	Leaves			Fruits			AgNPs		
	Viability (%)	Toxicity (%)	S.E.	Viability (%)	Toxicity (%)	S.E.	Viability (%)	S.E.	Toxicity (%)
500	6.633	93.367	0.00058	4.932	95.068	0.00033	6.803	0.00088	93.197
250	6.293	93.707	0.00033	5.102	94.898	0.00116	12.585	0.00353	87.415
125	5.952	94.048	0.00167	4.592	95.408	0.00058	15.476	0.00176	84.524
62.5	11.054	88.946	0.00186	6.293	93.707	0.00120	40.136	0.00176	59.863
31.25	20.918	79.081	0.00379	15.306	84.693	0.00416	53.401	0.00536	46.599
15.62	38.776	61.224	0.00458	23.809	76.190	0.00784	82.313	0.00318	17.687
7.81	49.489	50.510	0.00208	39.795	60.204	0.00379	97.959	0.00500	2.041
3.9	88.605	11.395	0.00328	82.143	17.857	0.00458	99.489	0.00452	0.510
0.00	100.000	0.000	0.00	100.000	0.000	0.00	100.000	0.00	0.000
IC <sub>50</sub>	11.440			8.80			47.320		

Table 2: Cytotoxicity of combined IC<sub>50</sub> of *J. procera* extract (leaves and fruits) with IC<sub>50</sub> AgNPs against Caco2 cells

Treatments	Concentration (µg mL <sup>-1</sup> )	Viability (%)	Toxicity (%)	Standard error
IC <sub>50</sub> Leaves+IC <sub>50</sub> AgNPs	11.440+47.32	34.60	65.40	0.00715
IC <sub>50</sub> Fruits+IC <sub>50</sub> AgNPs	8.800+47.32	25.56	74.44	0.00284
1/10 IC <sub>50</sub> Leaves+IC <sub>50</sub> AgNPs	1.144+47.32	52.54	47.46	0.00437
1/10 IC <sub>50</sub> Fruits+IC <sub>50</sub> AgNPs	0.880+47.32	59.37	40.64	0.00252
Control	0.00	100.00	0.00	0.00770

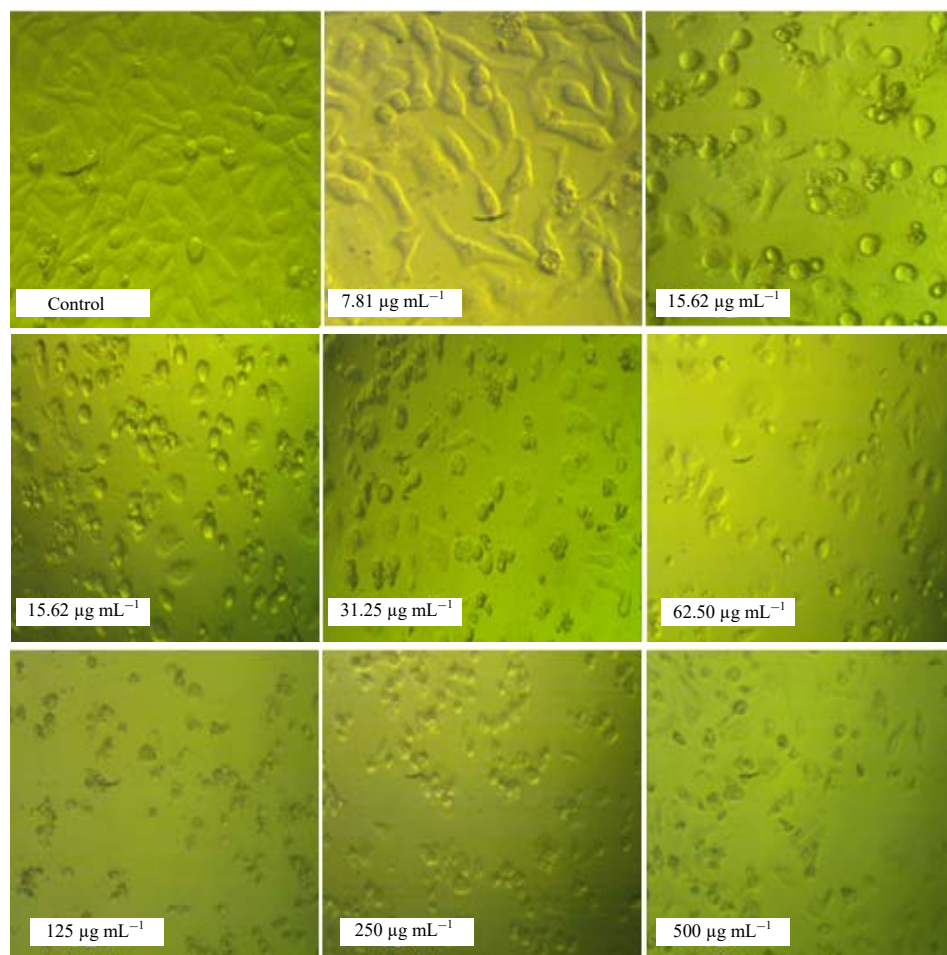


Fig. 1: Morphological features of Caco2 cells treated with different concentration of leaves extract of *J. procera*

Table 3: Nuclei counting of Caco2 cells lines treated with leaves, fruits extracts of *J. procera* and AgNPs

Concentration ( $\mu\text{g mL}^{-1}$ )	Number of nuclei		
	Leaves	Fruits	AgNPs
500	0	0	175000
250	25000	0	150000
125	50000	25000	475000
62.50	25000	50000	800000
31.25	75000	100000	1175000
15.62	275000	225000	1550000
7.81	500000	550000	1425000
3.90	1475000	1200000	1400000
0.00	1400000	1400000	1400000

### Effect of *J. procera* extract and AgNPs on DNA

**fragmentation of Caco2 cell line:** The DNA fragmentation assay was performed on Caco2 cells to elucidate the mechanism of cells death. As shown in Fig. 4, treatment with  $\text{IC}_{50}$  of fruit and leaves extract of *J. procera* (8.80 and  $11.44 \mu\text{g mL}^{-1}$ ) remained intact as in the controls and DNA smearing was not detected. In cells treated with  $\text{IC}_{50}$  of AgNPs light smearing was observed.

### DISCUSSION

The leaves and fruits extracts of *J. procera* displayed patented the inhibitory activity against Caco2 cells. Generally, *Juniperus* species are a good bet in the development of new drugs with natural compounds, it was reported that phytochemical analysis *J. procera* leaves indicated the presence of diterpenes, alkaloids and

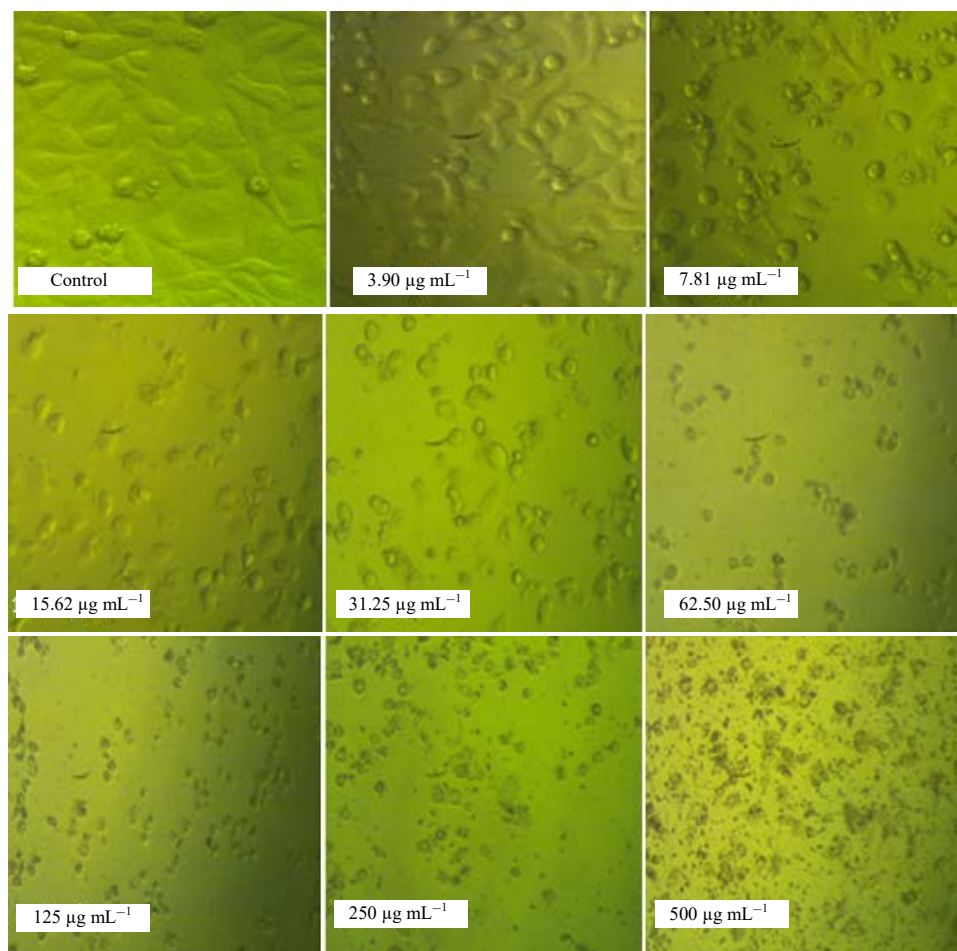


Fig. 2: Morphological features of Caco2 cells treated with different concentration of fruits extract of *J. procera*

flavonoids in extract. These constituents play an important role as anti-tumor activity. The  $IC_{50}$  obtained in current study on Caco2 cell using *J. procera* extract of fruits or leaves meaning that the extracts have potential anti-cancer properties<sup>42</sup>. Topcu *et al.*<sup>43</sup> showed weakly active ( $IC_{50} = 17.7 \mu\text{g mL}^{-1}$ ) of *J. excels* leaves extract against A2780 (human ovarian cancer cell line). In recent study, the anti-proliferative activity of *J. communis* berry extracts against Caco2 carcinoma cell line was noted, with  $IC_{50}$  value<sup>44</sup>  $500 \mu\text{g mL}^{-1}$ . In previous studies it was investigated that the anti-cancer activities of *J. procera* leaves extract against hepatocellular carcinoma (Hep G-2) cells lines with  $IC_{50}$  value  $131.9 \mu\text{g mL}^{-1}$ . The differences in  $IC_{50}$  may be due to species of plant, target cells, types of extracted solvent and ingredient of plant but all these factors don't eliminate effectiveness of *J. procera* against cancer cells<sup>42</sup>. Similar findings were reported by Nabi *et al.*<sup>29</sup>, where the anti-tumor activities of *J. excelsa* extract and its fractions showed good levels of

tumor inhibition 86.6% inhibition. AgNPs also showed anti-cancer activity against Caco2 cells (Table 1). Several studies reported that, good anti-cancer activity of AgNPs against HCT-116 colon cancer cells<sup>45</sup> and LoVo cells line<sup>46</sup>. Synergistic potential of AgNPs  $IC_{50}$  with leaves extract  $IC_{50}$  or of fruits extract  $IC_{50}$  were reported in the current study against cancer cells suggested that a better interaction of the mixture with tested cells. Generally, synergistic abilities of leaves and fruits extract of *J. procera* with AgNPs may reduce the use of AgNPs and therefore, reduce the side effects of metal.

Number of nuclei in treated cell decreasing with increasing extracts concentration. The toxic effect of treatments and its concentration reflected by the number of nuclei and suggestive for the apoptotic activities of treatments. Actually, the cytotoxic effect observed in current study is probably due to the presence of Hinokiol (3,12-dihydroxy-abieta-8,11,13-trien) whose presence in

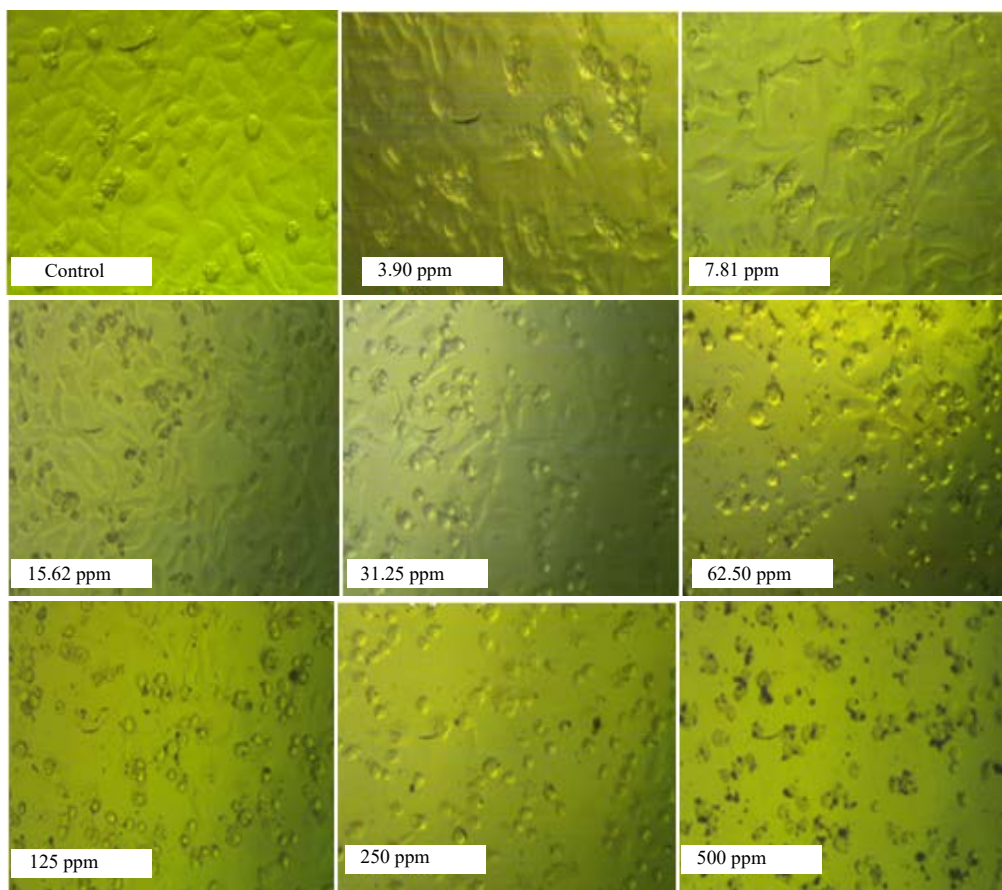


Fig. 3: Morphological features of Caco2 cells treated with different concentration of AgNPs

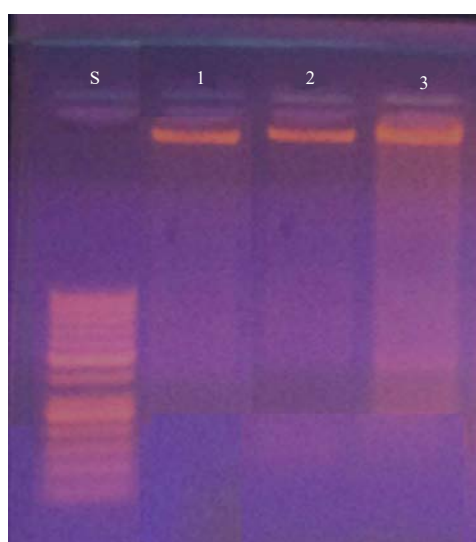


Fig. 4: Effect of leaves (Lane 1) and fruit (Lane 2) extract of *J. procera* and AgNPs (Lane 3) on DNA fragmentation in Caco2 cells. DNA standard 100 bp (Lane St.)

the *J. procera* has been reported by Wang *et al.*<sup>19</sup>. A very recent study detected that sugiol (2-hydroxy-abieta-8,11,13-triene-7-one) as a constituent in *J. procera* reduced the cell viability of human pancreatic cancer cells (Mia-PaCa2)<sup>47</sup>. Therefore, the results indicated that apoptosis may be induced by the use of these two plant extracts but further studies should be performed to confirm this conclusion. Compared to control cells, morphology of treated Caco2 cells were changed particularly at high concentrations strongly suggesting that cell death is occurring in the wells treated with the plant extract and AgNPs. George *et al.*<sup>48</sup> detected morphological alterations in the Caco2 cells exposed to *Rubus fairholmianus* root extract. Therefore, the results obtained from this study confirm that AgNPs induced cell death via apoptosis. The data obtained by Krishnaraj *et al.*<sup>49</sup> exhibited cytotoxic effects of AgNPs on MDA-MB-231, human breast cancer cells and the apoptotic features were confirmed through DNA fragmentation assays.



## CONCLUSION

These findings support and extend previous studies examining the anti-cancer effects of *J. procera* extracts against cancer cell. The *J. procera* fruits extract was more anti-cancer effective than leaves extract. The IC<sub>50</sub> of *J. procera* extracts of leaves or fruits induced the cytotoxic effects of AgNPs, therefore *J. procera* extract may minimize the concentration of AgNPs used in cancer treatment. The DNA fragmentation assay confirmed that AgNPs induced Caco2 cells death via apoptosis. The results indicated that *J. procera* was a promising anti-cancer agent for Caco2 cells. The performed experiments add scientific evidence to conduct further studies.

## SIGNIFICANCE STATEMENT

Public pressure to increase the use of natural therapeutic agent in treatment of diseases has increased in the recent years. Therefore; this study discovered the *J. procera* extract (leaves and fruits) for cancer treatment *in vitro*. This study will help the researchers to uncover the natural compounds from medicinal traditional plants that many researchers were not able to explore and helpful information in cancer therapy.

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## REFERENCES

1. Demir, S., Y. Aliyazicioglu, I. Turan, S. Misir and A. Mentese *et al.*, 2016. Antiproliferative and proapoptotic activity of *Turkish propolis* on human lung cancer cell line. *Nutr. Cancer*, 68: 165-172.
2. Rezaie-Tavirani, M., S. Fayazfar, S. Heydari-Keshel, M.B. Rezaee, M. Zamanian-Azodi, M. Rezaei-Tavirani and R. Khodarahmi, 2013. Effect of essential oil of *Rosa Damascena* on human colon cancer cell line SW742. *Gastroenterol. Hepatol. Bed Bench*, 6: 25-31.
3. Abdel Ghany, T.M., M. Ganash, M.M. Alawlaqi and A.M.H. Al-Rajhi, 2019. Antioxidant, antitumor, antimicrobial activities evaluation of *Musa paradisiaca* L. pseudostem exudate cultivated in Saudi Arabia. *BioNanoScience*, 9: 172-178.
4. Mishra, A., A. Seth and S.K. Maurya, 2016. Therapeutic significance and pharmacological activities of anti-diarrheal medicinal plants mention in Ayurveda: A review. *J. Intercult. Ethnopharmacol.*, 5: 290-307.
5. Akter, R., S.J. Uddin, I.D. Grice and E. Tiralongo, 2014. Cytotoxic activity screening of Bangladeshi medicinal plant extracts. *J. Nat. Med.*, 68: 246-252.
6. Bhalodia, N.R. and V.J. Shukla, 2011. Antibacterial and antifungal activities from leaf extracts of *Cassia fistula* L.: An ethnomedicinal plant. *J. Adv. Pharm. Technol. Res.*, 2: 104-109.
7. George, S., S.V. Bhalerao, E.A. Lidstone, I.S. Ahmad, A. Abbasi, B.T. Cunningham and K.L. Watkin, 2010. Cytotoxicity screening of Bangladeshi medicinal plant extracts on pancreatic cancer cells. *BMC Complement. Alternat. Med.*, Vol. 10. 10.1186/1472-6882-10-52.
8. Saetung, A., A. Itharat, C. Dechsukum, C. Wattanapiromsakul, N. Keawpradub and P. Ratanasuwan, 2005. Cytotoxic activity of Thai medicinal plants for cancer treatment. *Sonklanakar J. Sci. Technol.*, 27: 469-478.
9. Abdel Ghany, T.M., 2014. Eco-friendly and safe role of *Juniperus procera* in controlling of fungal growth and secondary metabolites. *J. Plant Pathol. Microbiol.*, Vol. 5. 10. 4172/2157-7471.1000231.
10. Abdel Ghany, T.M., M.A. Ganash, M. Marwah, M.H. Aisha and A. Mohamed, 2016. Evaluation of natural sources for repress cytotoxic Trichothecenes and Zearalenone production with using Enzyme-linked immunosorbent assay. *Life Sci. J.*, 13: 74-86.
11. Abdelghany, T.M., M.A. El-Naggar, M.A. Ganash and M.A. Al Abboud, 2017. PCR identification of *Aspergillus niger* with using natural additives for controlling and detection of malformins and maltoryzine production by HPLC. *BioNanoScience*, 7: 588-596.
12. Comte, G., D.P. Allais, A.J. Chulia, J. Vercauteren and C. Delage, 1996. Two furanone glucoside derivatives from *Juniperus phoenicea*. *Phytochemistry*, 41: 1329-1332.
13. Comte, G., D.P. Allais, A.J. Chulia, J. Vercauteren and N. Pinaud, 1997. Three phenylpropanoids from *Juniperus phoenicea*. *Phytochemistry*, 44: 1169-1173.
14. Stoilova, I.S., J. Wanner, L. Jirovetz, D. Trifonova, L. Krastev and A. Krastanov, 2014. Chemical composition and antioxidant properties of juniper berry (*Juniperus communis* L.) essential oil. *Bulgarian J. Agric. Sci.*, 20: 227-237.
15. Benzina, S., J. Harquail, S. Jean, A.P. Beauregard and C.D. Colquhoun *et al.*, 2015. Deoxydopodophyllotoxin isolated from *Juniperus communis* induces apoptosis in breast cancer cells. *Anti-Cancer Agents Med. Chem.*, 15: 79-88.
16. Ozturk, M., I. Tumen, A. Ugur, F. Aydogmus Ozturk and G. Topcu, 2011. Evaluation of fruit extracts of six Turkish *Juniperus* species for their antioxidant, anticholinesterase and antimicrobial activities. *J. Sci. Food Agric.*, 91: 867-876.
17. Saric-Kundali, B., C. Dobes, V. Klatt-Asselmeyer and J. Saukel, 2011. Ethnobotanical survey of traditionally used plants in human therapy of east, north and North-East Bosnia and Herzegovina. *J. Ethnopharmacol.*, 133: 1051-1076.

18. Samoylenko, V., D.C. Dunbar, M.A. Gafur, S.I. Khan and S.A. Ross *et al.*, 2008. Antiparasitic, nematocidal and antifouling constituents from *Juniperus* berries. *Phyther. Res.: Int. J. Devot. Pharmacol. Toxicol. Eval. Natural Prod. Deriv.*, 22: 1570-1576.
19. Wang, W.S., E.W. Li and Z.J. Jia, 2002. Terpenes from *Juniperus przewalskii* and their antitumor activities. *Die Pharm.*, 57: 343-345.
20. Ali, A.M., M.M. Mackeen, I. Intan-Safinar, M. Hamid, N.H. Lajis, S.H. El-Sharkawy and M. Murakoshi, 1996. Antitumor-promoting and antitumor activities of the crude extract from the leaves of *Juniperus chinensis*. *J. Ethnopharmacol.*, 53: 165-169.
21. Al-Attar, A.M., A.A. Alrobai and D.A. Almalki, 2016. Effect of *Olea oleaster* and *Juniperus procera* leaves extracts on thioacetamide induced hepatic cirrhosis in male albino mice. *Saudi J. Biol. Sci.*, 23: 363-371.
22. Muhammad, I., J.S. Mossa, M.A. Al Yahya, A.F. Ramadan and F.S. El Feraly, 1995. Further antibacterial diterpenes from the bark and leaves of *Juniperus procera* Hochst. ex Endl. *Phyther. Res.*, 9: 584-588.
23. Wu, M., Z. Jiang, H. Duan, L. Sun and S. Zhang *et al.*, 2013. Deoxypodophyllotoxin triggers necroptosis in human non-small cell lung cancer NCI-H460 cells. *Biomed. Pharmacoth.*, 67: 701-706.
24. Cairnes, D.A., O. Ekundayo and D.G. Kingston, 1980. Plant anti-cancer agents. X. Lignans from *Juniperus phoenicea*. *J. Natural Prod.*, 43: 495-497.
25. Hajar, D., S. Kremb, S. Sioud, A.H. Emwas, C.R. Voolstra and T. Ravasi, 2017. Anti-cancer agents in Saudi Arabian herbals revealed by automated high-content imaging. *Plos One*, Vol. 12. 10.1371/journal.pone.0177316.
26. McCabe, M., D. Gohdes, F. Morgan, J. Eakin, M. Sanders and C. Schmitt, 2005. Herbal therapies and diabetes among Navajo Indians. *Diabetes Care*, 28: 1534-1535.
27. Elmastas, M., I. Gulcin, S. Beydemir, O.I. Kufrevioglu and H.Y. Aboul-Enein, 2006. A study on the *in vitro* antioxidant activity of juniper (*Juniperus communis* L.) fruit extracts. *Anal. Lett.*, 39: 47-65.
28. Glisic, S.B., S.Z. Milojevic, S.I. Dimitrijevic, A.M. Orlovic and D.U. Skala, 2007. Antimicrobial activity of the essential oil and different fractions of *Juniperus communis* L. and a comparison with some commercial antibiotics. *J. Serb. Chem. Soc.*, 72: 311-320.
29. Nabi, S., N. Ahmed, M.J. Khan, Z. Bazai, M. Yasinzai and Y.M.S.A. Al-Kahraman, 2012. *In vitro* antileishmanial, antitumor activities and phytochemical studies of methanolic extract and its fractions of *Juniperus excelsa* berries. *World Applied Sci. J.*, 19: 1495-1500.
30. Abdel Ghany, T.M., 2013. *Stachybotrys chartarum*: A novel biological agent for the extracellular synthesis of silver nanoparticles and their antimicrobial activity. *Indonesian J. Biotechnol.*, 18: 75-82.
31. Abdel Ghany, T.M., A.R.M. Shater, M.A. Al Abboud and M.M. Alawlaqi, 2013. Silver nanoparticles biosynthesis by *Fusarium moniliforme* and their antimicrobial activity against some food-borne bacteria. *Mycopath*, 11: 1-7.
32. Abdelghany, T.M., A.M.H. Al-Rajhi, M.A. Al Abboud, M.M. Alawlaqi, A.G. Magdah, E.A.M. Helmy and S.M. Ahmed, 2018. Recent advances in green synthesis of silver nanoparticles and their Applications: About future directions. A review. *BioNanoSci.*, 8: 5-16.
33. Abdel-Ghany, T.M., M. Ganash, M.M. Bakri and A.M.H. Al-Rajhi, 2018. Molecular characterization of *Trichoderma asperellum* and lignocellulolytic activity on barley straw treated with silver nanoparticles. *BioResources*, 13: 1729-1744.
34. Ganash, M., T.A. Ghany and A.M. Omar, 2018. Morphological and biomolecules dynamics of phytopathogenic fungi under stress of silver nanoparticles. *BioNanoScience*, 8: 566-573.
35. Mittal, A.K., D. Tripathy, A. Choudhary, P.K. Aili, A. Chatterjee, I.P. Singh and U.C. Banerjee, 2015. Bio-synthesis of silver nanoparticles using *Potentilla fulgens* Wall. ex Hook. and its therapeutic evaluation as anti-cancer and antimicrobial agent. *Mater. Sci. Eng.: C*, 53: 120-127.
36. Migahid, A.M., 1974. Flora of Saudi Arabia. Cryptogams and Dicotyledons Equisetaceae to Neuradaceae. 4th Edn., Vol. 1, King Saud University Press, Riyadh, Saudi Arabia.
37. Chaudhary, A.C., 1997. Flora of the Kingdom of Saudi Arabia. Vol. 1, National Agriculture and Water Research Centre, Ministry of Agriculture, Saudi Arabia, Page: 691.
38. Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Methods*, 65: 55-63.
39. Zhu, Q., J. Meisinger, D.H. van Thiel, Y. Zhang and S. Mobarhan, 2002. Effects of soybean extract on morphology and survival of Caco-2, SW620 and HT-29 cells. *Nutr. Cancer*, 42: 131-140.
40. Chen, N., Z.M. Song, H. Tang, W.S. Xi, A. Cao, Y. Liu and H. Wang, 2016. Toxicological effects of Caco-2 cells following short-term and long-term exposure to Ag nanoparticles. *Int. J. Mol. Sci.*, Vol. 17. 10.3390/ijms17060974.
41. Abid-Essefi, S., I. Baudrimont, W. Hassen, Z. Ouanes and T.A. Mobio *et al.*, 2003. DNA fragmentation, apoptosis and cell cycle arrest induced by zearalenone in cultured DOK, Vero and Caco-2 cells: Prevention by vitamin E. *Toxicology*, 192: 237-248.
42. Abdel Ghany, T.M. and O.M. Hakamy, 2014. *Juniperus procera* as food safe additive, their antioxidant, anti-cancer and antimicrobial activity against some Food-borne bacteria. *J. Biol. Chem. Res.*, 31: 668-677.
43. Topcu, G., A.C. Goren, G. Bilsel, M. Bilsel, O. Cakmak, J. Schilling and D.G. Kingston, 2005. Cytotoxic activity and essential oil composition of leaves and berries of *Juniperus excelsa*. *Pharm. Biol.*, 43: 125-128.

44. Fernandez, A. and I.E. Cock, 2016. The therapeutic properties of *Juniperus communis* L.: Antioxidant capacity, bacterial growth inhibition, anti-cancer activity and toxicity. *Pharmacogn. J.*, 8: 273-280.
45. Mohanta, Y.K., S.K. Panda, R. Jayabalan, N. Sharma, A.K. Bastia and T.K. Mohanta, 2017. Antimicrobial, antioxidant and cytotoxic activity of silver nanoparticles synthesized by leaf extract of *Erythrina suberosa* (Roxb.). *Front. Mol. Biosci.*, Vol. 4. 10.3389/fmolb.2017.00014.
46. Mohammed, A.E., A. Al-Qahtani, A. Al-Mutairi, B. Al-Shamri and K. Aabed, 2018. Antibacterial and cytotoxic potential of biosynthesized silver nanoparticles by some plant extracts. *Nanomaterials*, Vol. 8. 10.3390/nano8060382.
47. Hao, C., X. Zhang, H. Zhang, H. Shang, J. Bao, H. Wang and Z. Li, 2018. Sugirol (12-hydroxyabieta-8, 11, 13-trien-7-one) targets hu-man pancreatic carcinoma cells (Mia-PaCa2) by inducing ap-optosis, G2/M cell cycle arrest, ROS production and inhibi-tion of cancer cell migration. *J. Buon*, 23: 205-210.
48. George, B.P.A., I.M. Tynga and H. Abrahamse, 2015. *In vitro* antiproliferative effect of the acetone extract of *Rubus fairholmianus gard.* root on human colorectal cancer cells. *BioMed Res. Int.*, Vol. 2015. 10.1155/2015/165037.
49. Krishnaraj, C., P. Muthukumaran, R. Ramachandran, M.D. Balakumaran and P.T. Kalaichelvan, 2014. *Acalypha indica* Linn: Biogenic synthesis of silver and gold nanoparticles and their cytotoxic effects against MDA-MB-231, human breast cancer cells. *Biotechnol. Rep.*, 4: 42-49.