Annals of R.S.C.B., ISSN: 1583-6258, Vol. 28, Issue 1, 2024, Pages. 13 - 26

Received 10 January 2024; Accepted 24 January 2024.

Comparison between the ability of ginger, pomegranate and curcumin to inhibit migration and invasion of U87 cells

ManarZraikat[1], Walhan Alshaer[2], Ali Zraikat[3], Suheil M. Zmeili[1]

Ebaa M Alzayadneh [4], Tasneem Alshelleh[1], Lana Koubaitary[1]

1Department of Pharmacology, School of Medicine, The University of Jordan, Amman,Jordan

2Cell Therapy Center, The University of Jordan

3 Department of Royal Medical services, Amman, Jordan

4Department of physiology , School of Medicine, The University of Jordan, Amman, Jordan

Corresponding author: Manar Zraikat. m.zraikat@ju.edu.jo Department of Pharmacology,

School of Medicine,The University of Jordan,Amman, Jordan. 11942

Abstract

Aim: Cancer is considered as one of the fatal diseases in most countries. Despite the high medical care development, most cancers are resistant to treatment. Therefore, there is a continuous research for novel treatment methods.

This study investigated the effect of curcumin, from *Curcuma longa*, different concentrations of soaked ginger and pomegranate juice on the invasion of U87 glioma cell line spheres in 3D collagen model. Furthermore, this study investigated the anti-migration effects of the mentioned plants on the migration of the same cell line in scratch assay.

Method: The Study was done in the department of Pharmacology, School of Medicine, The University of Jordan, Amman, Jordan. The 3D invasion assay and 2D migration assays were used to fulfill the mentioned aims in addition to the Image J program that was used to analyze the area of invasion and the area of migration over the days of applying the assays.

Results: Gradual effect of curcumin, the soaked ginger and Pomegranate juice was noticed on the inhibition of the invasion of U87 in collagen and on the inhibition of the migration of the same cell line in scratch assay in a dose and time-dependent manner. However, the results were more potent in migration compared to invasion.

Conclusion: This work adds more proofs on the importance of curcumin; ginger; and pomegranate juice as anti-invasive and anti-migration agents and opens the door for more investigative studies

Key words: Invasion, migration, Glioma, curcumin, Pomegranate

1. Introduction

Research targeting tumor have been enhanced in the last two decades. However, there has been no real advancements in investigating efficient methods to stop or hurdle cancer growth. It is always assumed that natural products would not have major harmful side effects compared with the available chemotherapeutic agents. The current problems associated with cancer chemotherapy resulted in a real shift toward natural alternatives for treatment of cancers (Bassiri-Jahromi 2018).

Received 10 January 2024; Accepted 24 January 2024.

Although cancer as a whole is considered as a major human health problem, the tumor dissemination has a special interest, as it is the major cause of death for most kinds of cancers (Talmadge and Fidler 2010).The difficulty in developing drugs that target controlling cancer is mainly due to tumor dissemination once it happens, in addition to the development of drug resistance by cancer cells (Glickman and Sawyers 2012).

Among the best strategies to fight cancer is by fighting its metastases, however, these strategies have not yet entered routine clinical care, because of the lack of clinical validation studies. In this context, further studies are needed to explore potential preventive agents that could be helpful in preventing metastatic growth. The prevention of metastasis could be on three levels. The first level includes preventing the cancer before it ever occurs by reducing exposure to risk factors. The second level includes reducing the effects and complications of cancer that already started. This is done by offering the needed treatment before the progression of cancer at the initiation stage of tumor. The third stage involves improving the ability to manage long term health problems associated with established tumors to limit its invasion and hopeful increase in life expectancy.

Tumor dissemination is a process that involves different definitions including invasion, migration, adhesion, metastasis and angiogenesis. Invasion is considered one of the most important steps in tumor dissemination process that includes alterations of many proteins (Barkan, Green et al. 2010). Controlling the invasion and migration of the tumor is considered a key issue in the control of the whole dissemination process. This paper investigates the preventive effect of three natural plants against cancer invasion and migration. These plants include; *Curcuma longa, or* turmeric, Ginger, or *Zingiber officinale Roscoe*, or *Zingiberaceae, and* Pomegranate (Punica granatum L).

Curcuma longa, or turmeric, is the main source of curcumin which is known as the polyphenol Curcumin. Turmeric is well known and widely used oriental food spice. Recently, curcumin is well documented to have antioxidant effect in Indian and Chinese medicine (Chainani-Wu and Medicine 2003) According to new studies, curcumin was found to have antiproliferative properties in vitro (Mehta, Pantazis et al. 1997). The interference with tumor cell cycle is supposed to be behind the curcumin properties of suppression of cancer cell growth (Schwertheim, Wein et al. 2017). Furthermore, curcumin has anti invasion properties due to its effect in regulation of growth factors and adhesion molecules (Killian, Kronski et al. 2012).

Ginger, or *Zingiber officinale Roscoe*, or *Zingiberaceae* is known as one of famous herbs in traditional medicine in addition to its use as flavoring agent. Inflammatory diseases have been dealt with by ginger for long time in traditional medicine (Afzal, Al-Hadidi et al. 2001). The anti-inflammatory, antioxidant and anti-cancer properties of ginger are supposed to be due to the presence of active ingredients such as phenolic compounds including shogaol, gingerol, and paradol (Jeyakumar, Nalini et al. 1999),(Shukla, Singh et al. 2007),(Huang, DeGuzman et al. 2000) (Oyagbemi, Saba et al. 2010), (Shukla, Singh et al. 2007), (Baliga, Haniadka et al. 2011) (Lee, Lee et al. 2009), (Lee, Seo et al. 2008), (Lee, Cekanova et al. 2008), (Brown, Shah et al. 2009), (Weng, Wu et al. 2010), (Kim, Lee et al. 2014), (Fan, Yang et al. 2015), (Kim, Im Lee et al. 2008), (Sang, Hong et al. 2009)). On molecular level, ovarian cancer cells treated by ginger extract have shown down-regulation of NF-κB-regulated gene products involved in cellular proliferation and angiogenesis, including IL-8, 20 and VEGF21 (Coppola and Novo 2007). Furthermore, treating breast cancer cells with gingerol resulted in inhibiting cell proliferation due to presence of 10-gingerol which resulted in inhibiting mitogen-induced Akt and p38MAPK activation and EGFR expression (Joo, Hong et al. 2016) The effect of ginger active ingredients such as gingerol and shogaol is extended to inhibit the invasion of breast cancer cells through down regulating MMP-2 and MMP-9 metalloproteinases which are known to be involved in inducing cancer cell invasion (Weng, Chou et al. 2012), (Seo, Li et al. 2003) .

Pomegranate (Punica granatum L.) is a round fruit with an outer hard shiny skin and an inner purple to reddish seeds that belongs to *Punicaceae* family. This fruit is widely consumed as raw seeds or as a juice by many people all over the world. Pomegranate has important medical history, and valuable medicinal properties (Bassiri-Jahromi 2018). Pomegranate is among the natural sources, which has shown to have an anti-proliferative and an anti-cancer effects against different cancer types such as breast, prostate, colon, and lung cancers (Longtin 2003).

Received 10 January 2024; Accepted 24 January 2024.

In addition to the anticancerous effect of pomegranate, it has been shown to have bioactive properties and an antioxidant activity (Sharma, McClees et al. 2017). Pomegranate is considered as a very important source of polyphenolics and tannin (Amakura, Okada et al. 2000). Furthermore, the pomegranate peels contain active inhibitors, such as flavonoids and phenolics (Al-Zoreky 2009). Practically, Pomegranate was found to be active against oxidative damage in diabetic rats (Longtin 2003). Pomegranate has also shown to have an anti-invasive, anti-proliferative, anti-cancerous and anti-metastatic effects in vitro and in vivo on different cancer cell line (Amakura, Okada et al. 2000)**.**

This work aims to compare the effect of curcumin, ginger and pomegranate fresh juice on the invasion of U87 glioma cell lines in 3D spheroid invasion model. Furthermore, this work aims to compare their effect on the migration of the same cell line in 2D scratch model. Finally, this work aims to compare the anti-invasion and anti-migration properties of these natural products in both the 3D invasion assay and the 2D migration assay.

2. Material and methods

Curcumin is the generic name for (E,E)-1,7-bis(4-Hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, Diferuloylmethane, Pure curcumin powder from *Curcuma longa* was purchased from Sigma- Aldrich, USA.

Ginger was purchased from the local market. Small pieces of the ginger root were soaked (*Zingiber officinale*) in the tissue culture water over-night. The soak was filtered by 0.45 um filters and aliquotted into 200ul aliquots and stored in -20 freezer until used.

Fresh Pomegranate juice was prepared from crushed pomegranate seeds without the peal. The freshly prepared juice was filtered using 0.45 filters and aliquotted then stored at -20C until used later.

The U87-MG Glioma cell line was purchased from the European Collection of Cell Cultures ((ECACC), Salisbury, Wiltshire, England). The cells were maintained in full RPMI 1640 medium and cultured at 37°C in a 5% CO2 humidified atmosphere. Collagen I was purchased from Sigma-Aldrich (Poole, UK). Collagen I with catalogue number C4243 and all MTT assay reagents were purchased from Sigma-Aldrich (Poole, UK).

After treating the cells with different concentrations of curcumin, Ginger and Pomegranate juice the cells were incubated with MTT at 37°C, 5% CO2 for 4 hrs. MTT solution was removed and the optical density of the plates was read at 550 nm. The MTT Cytotoxicity assay was repeated three times. The MTT stock solution was prepared in 5 mg/ml concentrations and diluted to a final concentration of 0.5mg/ml.

For Collagen invasion assay; The U87 spheroids were prepared by the hanging drop method and seeded in 8-chamber cover glass (Nunc, Lab-TeK, Thermo Scientific) between two layers of Collagen I (pH 7.4). The assay was incubated at 37°C, 5% CO2 for 7 days with daily images captured using inverted light microscopy. The spheroid invasion area was analyzed using ImageJ program.

The scratch assay was done after the U87cells reached 70 -80 % confluency. The curcumin Ginger and Pomegranate juice were added after the cells were washed. The scratch area was analyzed using ImageJ program which was used to analyze representative pictures of the scratch over the first 48 hours. Student-test analysis was used to analyze the data. Results were considered statistically significant with p value = 0.05.

3. Results

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 28, Issue 1, 2024, Pages. 13 - 26

Received 10 January 2024; Accepted 24 January 2024.

MTT assay was done for the U87 cells after treatment with different concentrations of curcumin, pomegranate juice and ginger soak and repeated three times. The IC50 for curcumin was around 264.4 uM, the IC50 of pomegranate juice was around 18% (v/v) and the IC50 for ginger was around 30% (v/v). The concentrations used in the 3D invasion assay and the scratch assay were chosen to be less than IC50 for all natural products

The collagen invasion assay was performed on different concentrations of curcumin, pomegranate juice and ginger soak. In relation to curcumin, the concentration round 6uM showed relatively no effect on the invasion of the U87 cells compared to the control. With the concentration of 63.3uM, which is around one fourth of the IC50, there was significant inhibition of the invasion of the U87 spheres in collagen (Figure1, 7).

The effect of ginger soak on the invasion of U87 spheres in collagen was investigated through examining different concentrations in 3D invasion assay. Gradual inhibition of U87 spheres invasion was noticed after examining gradual concentrations of ginger soak (Figure4, 7). In the first two concentrations, the effect of ginger was seen through not having continued increase in the size of the invasion after end of the 48 hours as seen in the control (Figure 4, 7). The concentration of 25% (v/v) has shown a significant inhibition of the U87 spheres invasion (Figure 4, 7).

In relation to pomegranate juice the 3D invasion assay was repeated three times using three different concentrations, which are selected to be less than the IC50 as much as possible. The three concentrations were; 1.7% (v/v), 3.3%(v/v) and 5% (v/v). The different concentrations of pomegranate juice showed a gradual inhibition of U87 spheres invasion in collagen as compared to the control (Figures). The results were statistically significant with p values between 0.05 and $p < 0.1$.

Different concentrations of less than the IC50 were tested to determine the effect of curcumin, pomegranate juice and ginger soak on the migration of U87 cell in scratch assay. In relation to curcumin, the concentration of 8.4 uM had comparable effect to the control. However, starting from the concentration of 35 uM, - a about one eighth of the IC50, a gradual increase in the inhibition of U87 cell in scratch assay was noticed (Figure).

In relation to ginger soak a gradual ability to inhibit the migration of the U78 cells was noticed over the first 4 hours after treatment. The concentration of 0.66% (v/v) of ginger soak was comparable to the control in relation to the cell migration. However starting from the concentration 1%, a significant gradual inhibition of migration was noticed. The concentrations 2% and 33.3% were comparable in their ability to inhibit the migration of the U87 cells in the scratch assay after 48 hours of treatment; (Figure).

In relation to the pomegranate juice the scratch assay was repeated three times using different concentrations. The results showed a gradual inhibition of the U87 cells migration. The effect was noticed on concentrations less than the concentrations used in the 3 D assay (Figures).

4. Discussion

The 3D spherical invasion assay was used to investigate the effect of curcumin, pomegranate juice and ginger soak on the invasion of U87 cells. This is because this assay is considered as a representative model to the tumour in vivo. The 3D structure of the spheres is representative of 3D tumour mass in addition to the collagen layers which are representative of tumour microenvironment. Collagen makes a barrier layer between the drug and the tumour mass making this model more challenging in relation to drug delivery. The presence of collagen makes the invasion process more challenging and more able to mimic the in vivo situation. The 3D structure of the spheres makes the model able to mimic as much as possible the 3D structure of the in vivo tumors compared to the 2 D models in general.

Received 10 January 2024; Accepted 24 January 2024.

Gliomas are the most frequent invasive malignant tumors of the brain, and glioma cell lines are commonly used in research to assess anti cancerous effect of drugs and their therapeutic application in terms of tumor growth, invasion, migration and angiogenesis (Remondelli and Renna 2017) (Giakoumettis, Kritis et al. 2018).

When comparing the 3D invasion assay and the 2D migration assay, the inhibitory effect of curcumin, pomegranate juice and ginger soak was seen in lower concentrations in 2D compared to the 3D model. These results explain why the 3 D model is more challenging and representative of *in vivo* conditions.

Curcumin has shown a significant inhibitory effect on the invasion and the migration of the U87 cells. This effect could be explained by many publications which showed that curcumin has an inhibitory effect on the expression of MMPs (Swarnakar, Ganguly et al. 2005), (Shakibaei, John et al. 2007) in inflammatory diseases and many cell lines (Mitra, Chakrabarti et al. 2006) (Su, Chen et al. 2006) (Hong, Ahn et al. 2006) (Lin, Ke et al. 1998) The inhibitory effect of curcumin could be due to its effect in down- regulating the expression of Ap-1 and NF_B (Bachmeier, Nerlich et al. 2007) (Kim, Noh et al. 2012).

In relation to ginger soak there had been a gradual inhibition of U87 cells invasion and migration using the 3D invasion assay and 2 D scratch assay. The ginger soak concentration of 25% had shown a significant inhibition of the invasion of the U87 spheres. The inhibitory effect of ginger was seen with lower concentrations in the 2 D migration assay compared to the concentrations used in the 3 D invasion assay. This effect could be due to different factors. First, the presence of collagen in the 3 D invasion assay exerts a challenging barrier between the drug and the cells compared to the 2 D scratch assay, which has no barriers. Second, the presence of the cells in 3D spherical shape in the 3D invasion assay, render the delivery of the drug to cells more challenging compared to the 2D migration assay, where the cells are in 2D layer.

Pomegranate fruit, a popular constituent of healthy diet, is cultivated in many areas of the world particularly in the Mediterranean region (Lansky, Shubert et al. 2000). The beneficial medical benefits of all parts of pomegranate fruit were known for thousands of years due to its high content of vitamin C and antioxidants. For example, it was used by many people in the management of diarrhea, sore throat, peptic ulcer, osteoarthritis, heart diseases and diabetes mellitus (Lansky, Shubert et al. 2000) (Ismail, Sestili et al. 2012) (Colombo, Sangiovanni et al. 2013). Other traditional uses of pomegranate products have included hypertension, fertility aid, intestinal bacterial infection, intestinal inflammatory diseases as Crohn's disease, intestinal helminthes infestations and hemorrhage (Lansky, Shubert et al. 2000). In addition to the wide range of traditional clinical uses of pomegranate, it has been found that pomegranate juice, peel and oil have anticancerous activities, including interference with tumor cell proliferation, cell cycle, invasion and angiogenesis (Bassiri-Jahromi 2018). It is believed that the anti-cancerous effects to pomegranate are mainly attributed to its high anti-inflammatory and antioxidant properties (Lansky and Newman 2007). The results have shown gradual effect of pomegranate juice on the inhibition of U87 invasion and migration. The pomegranate juice has been more efficient in inhibiting the migration of the cells compared to its effect on the invasion. This effect is mostly because the 3D invasion assay is more challenging than the 2D migration assay.

5. Conclusion

Curcumin has shown an inhibitory effect on the invasion and migration of U87 glioma cells. The inhibitory effect of curcumin was gradual and started on concentrations much less than the IC 50. Further future studies are needed to investigate this effect of curcumin on the molecular level, and to have an idea about its mechanism of action in inhibiting malignant cell growth. The ginger soak could inhibit the migration of U87 glioma cells on lower concentrations compared to those concentrations that inhibited the invasion in the 3D assay. This could be due to the more challenging properties of the 3D assay. The pomegranate juice is considered as promising agent in the field of Glioma treatment research that needs more investigation and analysis to characterize the active ingredients that are behind its anti-invasive and anti-migration effects.

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 28, Issue 1, 2024, Pages. 13 - 26

Received 10 January 2024; Accepted 24 January 2024.

6. Acknowledgements

This work was financially supported by the dean of scientific research in the University of Jordan.

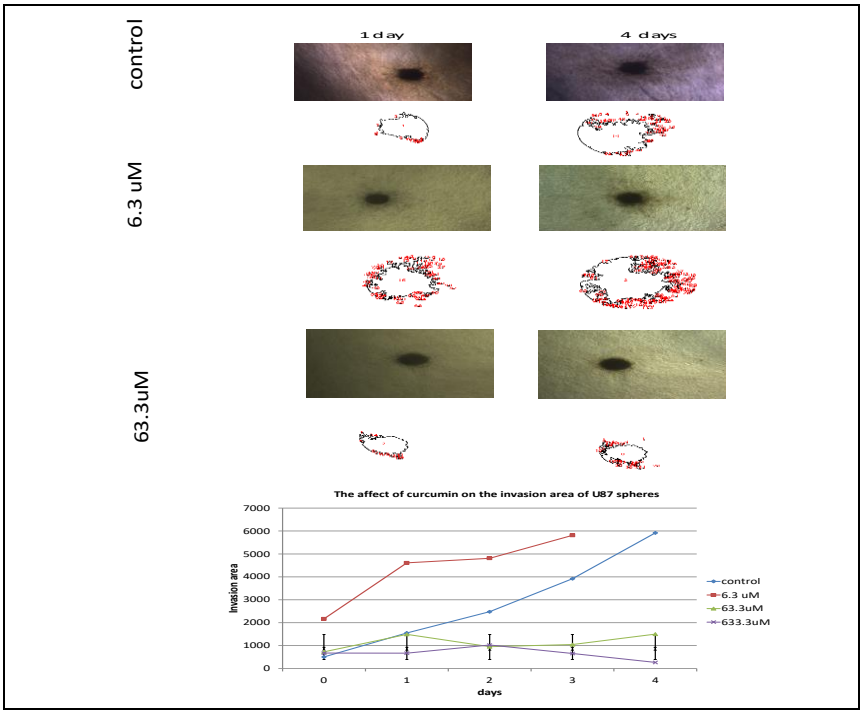


Figure1. The effect of different concentrations of curcumin on the invasion of U87 cells in 3D invasion assay and diagram showing the relation between the invasion area and time.

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 28, Issue 1, 2024, Pages. 13 - 26

Received 10 January 2024; Accepted 24 January 2024.

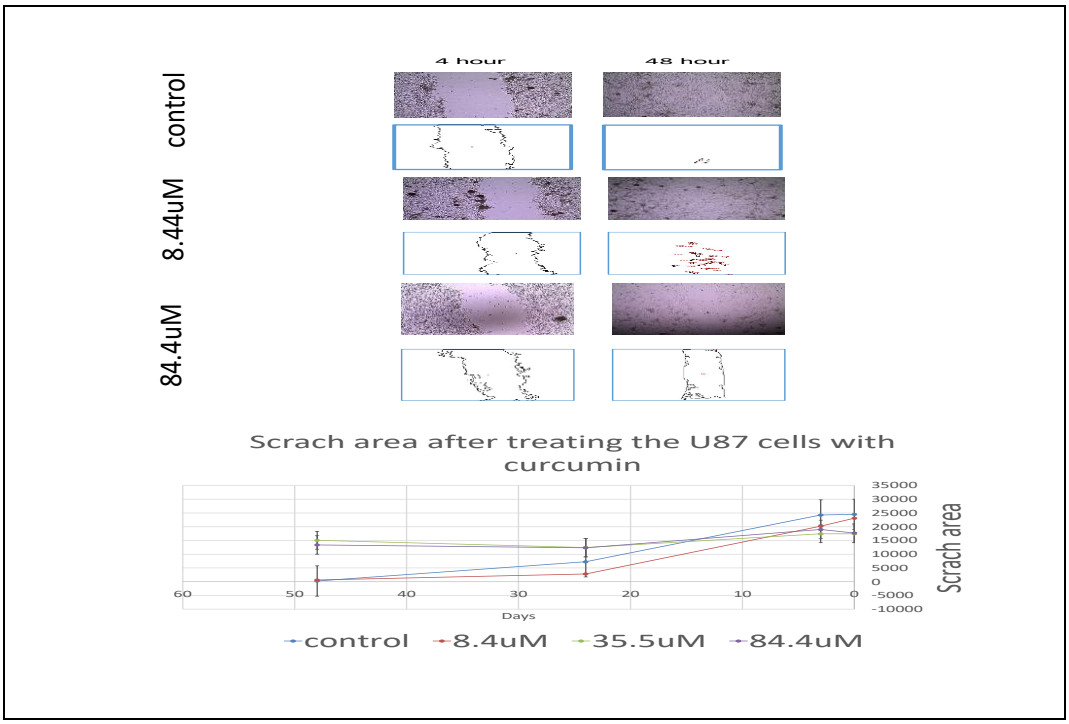


Figure 2. The effect of different concentrations of curcumin on the migration area of the U87 cells in scratch assay and diagram shows the relation between the migration area and Time in the scratch assay.

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 28, Issue 1, 2024, Pages. 13 - 26

Received 10 January 2024; Accepted 24 January 2024.

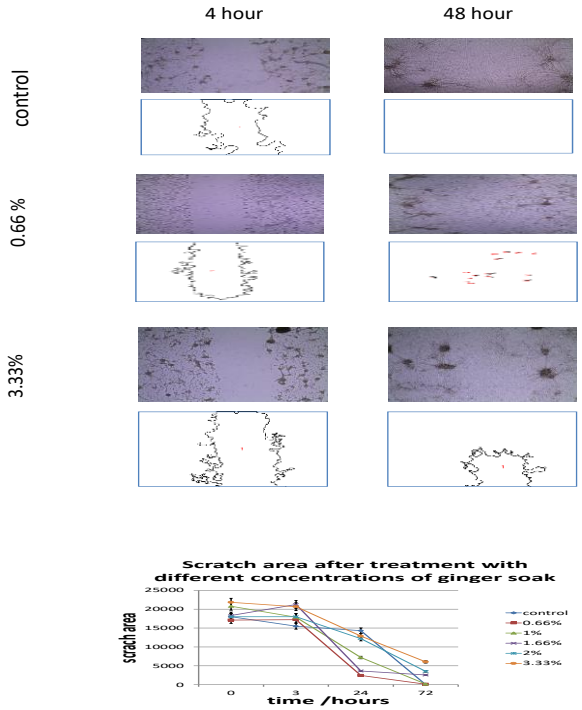


Figure 3: Scratch assay after treating U87 cells with different concentrations of ginger soak indicating a gradual inhibition of the cell migration after 48 h of treatment

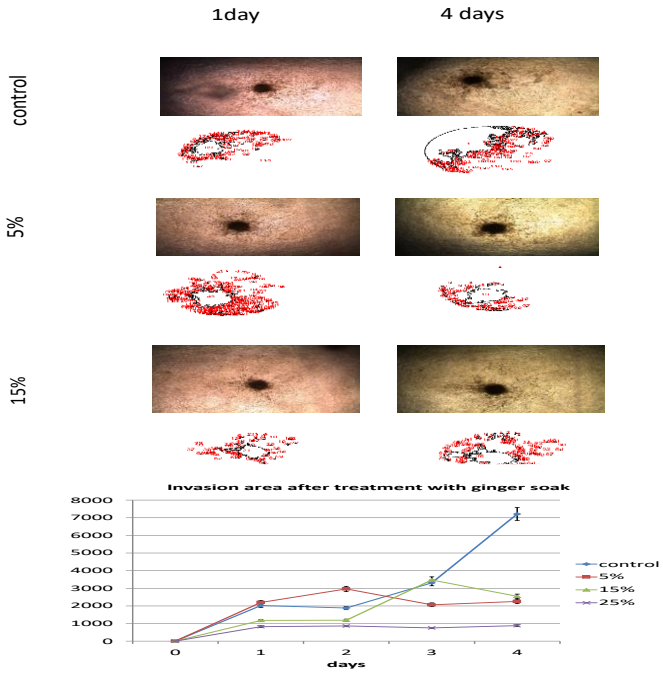


Figure 4: Effect of different concentrations of ginger soak on the invasion of U87 spheres in collagen. Gradual inhibition of U87 invasion by different concentrations of ginger soak.

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 28, Issue 1, 2024, Pages. 13 - 26

Received 10 January 2024; Accepted 24 January 2024.

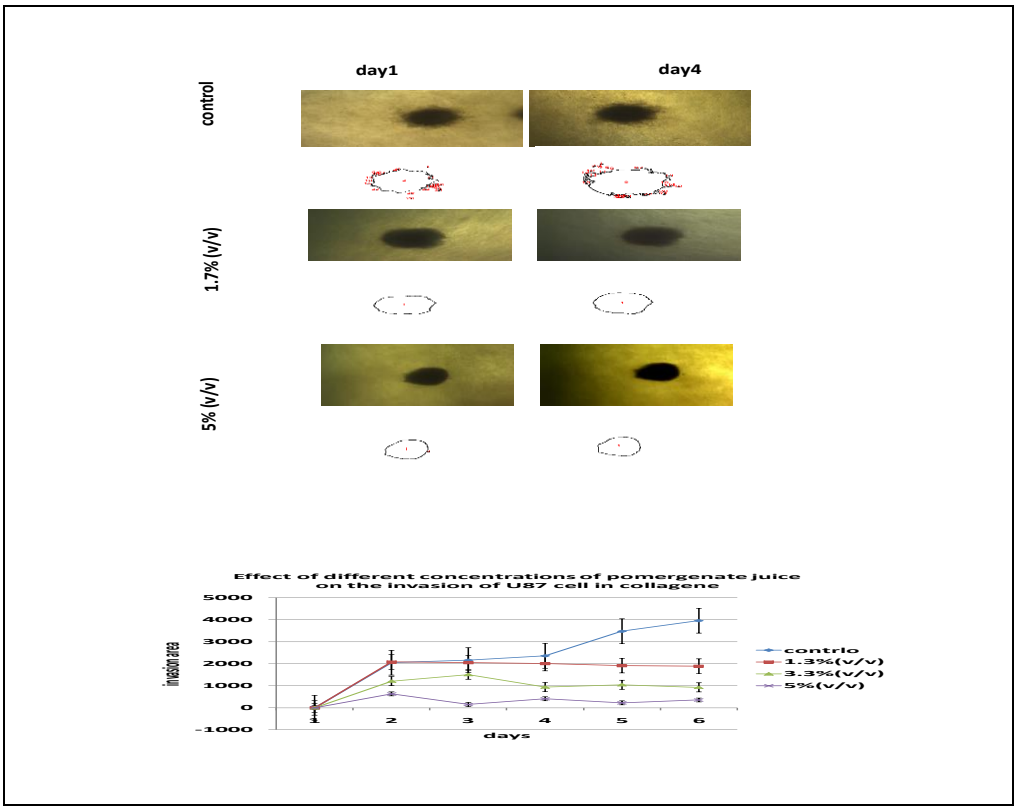
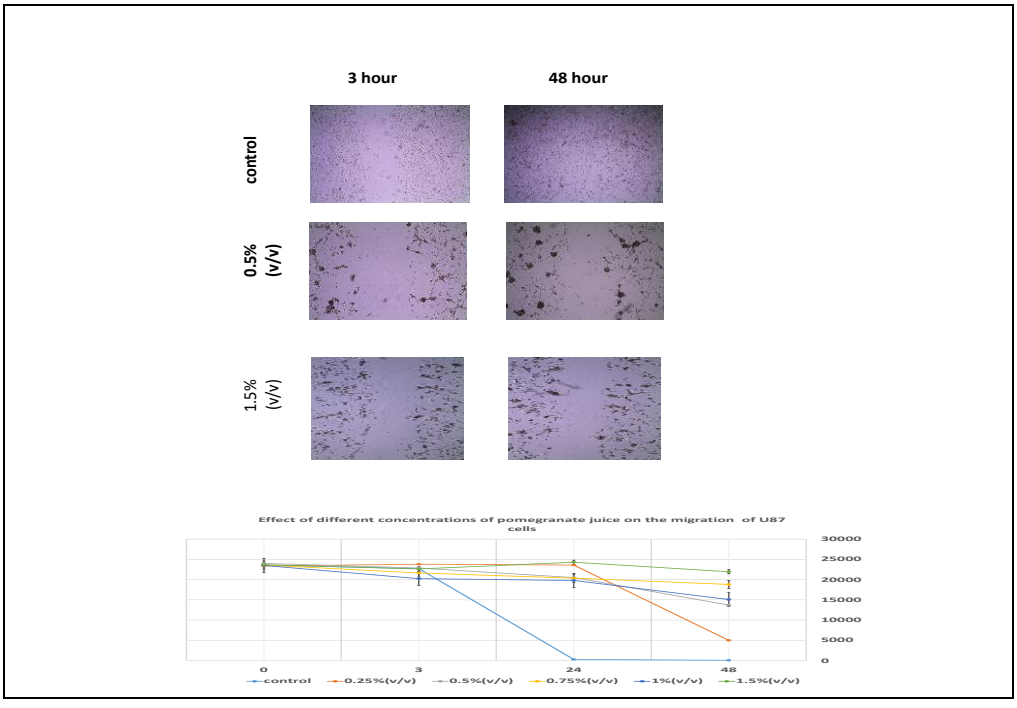


Figure 5. Gradual inhibition of U87 spheres invasion in collagen compared to the control. The effect of different concentrations of pomegranate juice on the invasion of U87 spheres.



Annals of R.S.C.B., ISSN: 1583-6258, Vol. 28, Issue 1, 2024, Pages. 13 - 26

Received 10 January 2024; Accepted 24 January 2024.

Figure 6. Gradual inhibition of U87 migration using different concentrations of pomegranate juice. The effect of different concentrations of pomegranate juice on the inhibition of U87 cell migration.

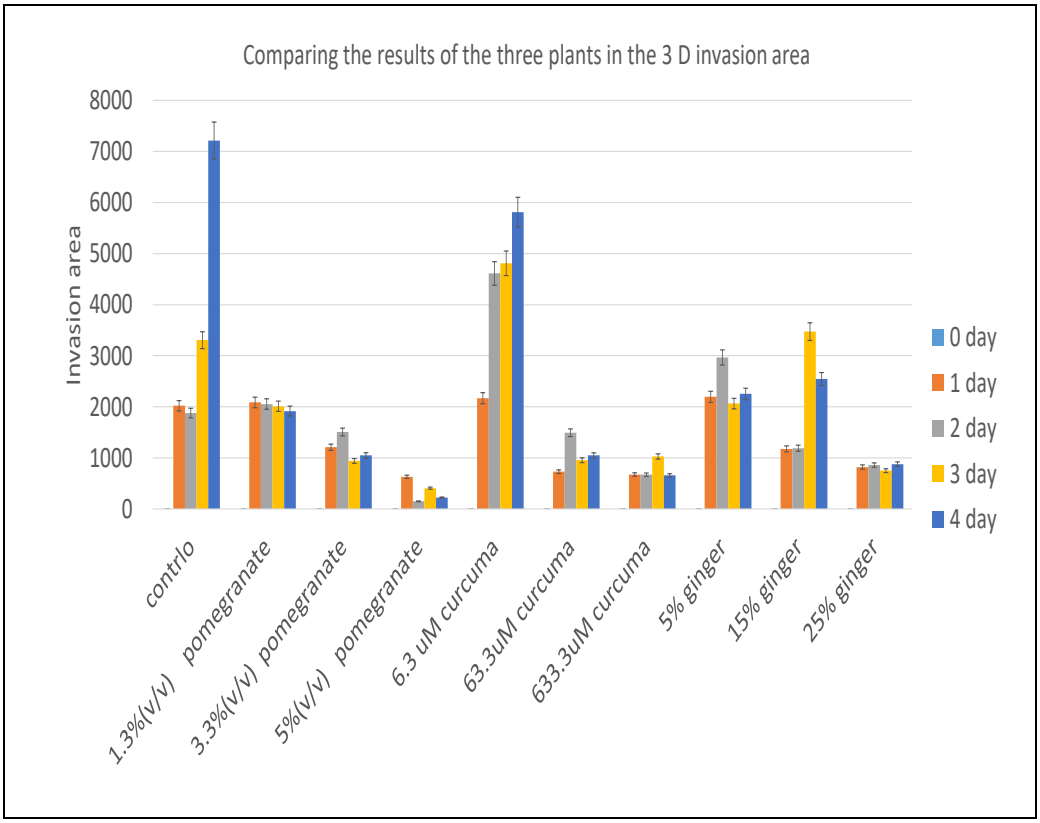


Figure 7 Comparison between the three plants in the 3 D invasion assay. The results show the high efficacy of pomegranate compared to the ginger and curcuma.

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 28, Issue 1, 2024, Pages. 13 - 26

Received 10 January 2024; Accepted 24 January 2024.

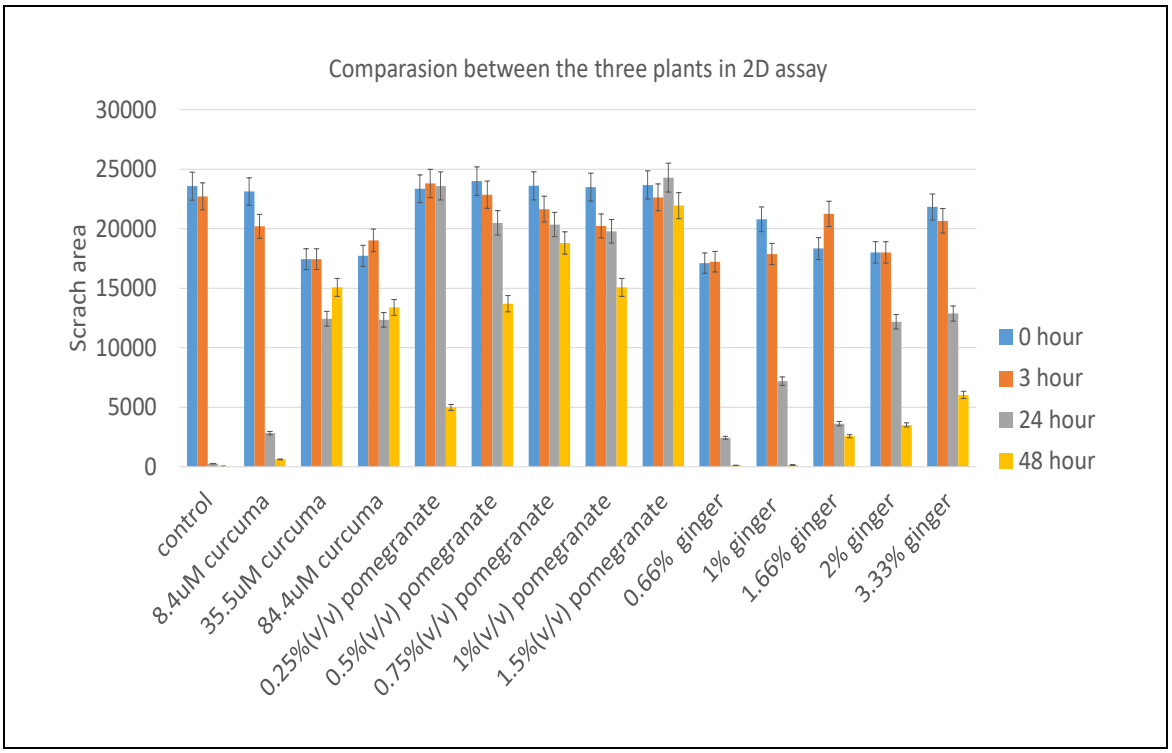


Figure 8: Comparison between the three plants in the 2 D scrach assay. The results show the high efficacy of pomegranate compared to the ginger and curcuma.

References

1. Afzal, M., D. Al-Hadidi, M. Menon, J. Pesek, M. J. D. m. Dhami and d. interactions (2001). "Ginger: an ethnomedical, chemical and pharmacological review." **18**(3-4): 159-190.
2. Al-Zoreky, N. (2009). "Antimicrobial activity of pomegranate (Punica granatum L.) fruit peels." International journal of food microbiology **134**(3): 244-248.
3. Amakura, Y., M. Okada, S. Tsuji and Y. Tonogai (2000). "High-performance liquid chromatographic determination with photodiode array detection of ellagic acid in fresh and processed fruits." Journal of Chromatography A **896**(1-2): 87-93.
4. Amakura, Y., M. Okada, S. Tsuji and Y. J. J. o. C. A. Tonogai (2000). "High-performance liquid chromatographic determination with photodiode array detection of ellagic acid in fresh and processed fruits." **896**(1-2): 87-93.
5. Bachmeier, B., A. Nerlich, C. Iancu, M. Cilli, E. Schleicher, R. Vené, R. Dell'Eva, M. Jochum, A. Albini, U. J. C. p. Pfeffer and biochemistry (2007). "The chemopreventive polyphenol Curcumin prevents hematogenous breast cancer metastases in immunodeficient mice." **19**(1-4): 137-152.
6. Baliga, M. S., R. Haniadka, M. M. Pereira, J. J. D'Souza, P. L. Pallaty, H. P. Bhat, S. J. C. r. i. f. s. Popuri and nutrition (2011). "Update on the chemopreventive effects of ginger and its phytochemicals." **51**(6): 499-523.

Received 10 January 2024; Accepted 24 January 2024.

7. Barkan, D., J. E. Green and A. F. Chambers (2010). "Extracellular matrix: a gatekeeper in the transition from dormancy to metastatic growth." European journal of cancer **46**(7): 1181-1188.
8. Bassiri-Jahromi, S. (2018). "Punica granatum (Pomegranate) activity in health promotion and cancer prevention." Oncology reviews **12**(1).
9. Bassiri-Jahromi, S. J. O. r. (2018). "Punica granatum (Pomegranate) activity in health promotion and cancer prevention." **12**(1).
10. Brown, A. C., C. Shah, J. Liu, J. T. Pham, J. G. Zhang, M. R. J. P. R. A. I. J. D. t. P. Jadus and T. E. o. N. P. Derivatives (2009). "Ginger's (Zingiber officinale Roscoe) inhibition of rat colonic adenocarcinoma cells proliferation and angiogenesis in vitro." **23**(5): 640-645.
11. Chainani-Wu, N. J. T. J. o. A. and C. Medicine (2003). "Safety and anti-inflammatory activity of curcumin: a component of tumeric (Curcuma longa)." **9**(1): 161-168.
12. Colombo, E., E. Sangiovanni, M. J. E.-B. C. Dell'Agli and A. Medicine (2013). "A review on the anti-inflammatory activity of pomegranate in the gastrointestinal tract." **2013**.
13. Coppola, G. and S. J. A. o. m. r. Novo (2007). "Statins and peripheral arterial disease: effects on claudication, disease progression, and prevention of cardiovascular events." **38**(5): 479-488.
14. Fan, J., X. Yang and Z. J. T. B. Bi (2015). "6-Gingerol inhibits osteosarcoma cell proliferation through apoptosis and AMPK activation." **36**(2): 1135-1141.
15. Giakoumettis, D., A. Kritis and N. J. H. Foroglou (2018). "C6 cell line: the gold standard in glioma research." **22**(3): 105.
16. Glickman, M. S. and C. L. Sawyers (2012). "Converting cancer therapies into cures: lessons from infectious diseases." Cell **148**(6): 1089-1098.
17. Hong, J., K. Ahn, E. Bae, S. Jeon, H. J. P. c. Choi and p. diseases (2006). "The effects of curcumin on the invasiveness of prostate cancer in vitro and in vivo." **9**(2): 147-152.
18. Huang, S., A. DeGuzman, C. D. Bucana and I. J. J. C. C. R. Fidler (2000). "Nuclear factor-κB activity correlates with growth, angiogenesis, and metastasis of human melanoma cells in nude mice." **6**(6): 2573-2581.
19. Ismail, T., P. Sestili and S. J. J. o. e. Akhtar (2012). "Pomegranate peel and fruit extracts: a review of potential anti-inflammatory and anti-infective effects." **143**(2): 397-405.
20. Jeyakumar, S., N. Nalini and V. P. J. M. s. r. Menon (1999). "Antioxidant activity of ginger (Zingiber officinale Rosc) in rats fed a high fat diet." **27**(5): 341-344.
21. Joo, J.-H., S.-S. Hong, Y.-R. Cho and D.-W. J. O. r. Seo (2016). "10-Gingerol inhibits proliferation and invasion of MDA-MB-231 breast cancer cells through suppression of Akt and p38MAPK activity." **35**(2): 779-784.
22. Killian, P. H., E. Kronski, K. M. Michalik, O. Barbieri, S. Astigiano, C. P. Sommerhoff, U. Pfeffer, A. G. Nerlich and B. E. J. C. Bachmeier (2012). "Curcumin inhibits prostate cancer metastasis in vivo by targeting the inflammatory cytokines CXCL1 and-2." **33**(12): 2507-2519.
23. Kim, J.-M., E.-M. Noh, K.-B. Kwon, J.-S. Kim, Y.-O. You, J.-K. Hwang, B.-M. Hwang, B.-S. Kim, S.-H. Lee and S. J. J. P. Lee (2012). "Curcumin suppresses the TPA-induced invasion through inhibition of PKCα-dependent MMP-expression in MCF-7 human breast cancer cells." **19**(12): 1085-1092.
24. Kim, J. S., S. Im Lee, H. W. Park, J. H. Yang, T.-Y. Shin, Y.-C. Kim, N.-I. Baek, S.-H. Kim, S. U. Choi and B.-M. J. A. o. p. r. Kwon (2008). "Cytotoxic components from the dried rhizomes of Zingiber officinaleRoscoe." **31**(4): 415.

25. Kim, M. O., M.-H. Lee, N. Oi, S.-H. Kim, K. B. Bae, Z. Huang, D. J. Kim, K. Reddy, S.-Y. Lee and S. J. J. C. Park (2014). "[6]-Shogaol inhibits growth and induces apoptosis of non-small cell lung cancer cells by directly regulating Akt1/2." **35**(3): 683-691.
26. Lansky, E., S. Shubert, I. J. P. Neeman, Processing, M. o. P. i. t. M. R. A. i. Research and s. A. S. M. Technology. Options Méditerranéennes (2000). "Pharmacological and therapeutic properties of pomegranate." **42**: 231-235.
27. Lansky, E. P. and R. A. J. J. o. e. Newman (2007). "Punica granatum (pomegranate) and its potential for prevention and treatment of inflammation and cancer." **109**(2): 177-206.
28. Lee, H. S., E. Y. Seo, N. E. Kang and W. K. J. T. J. o. n. b. Kim (2008). "[6]-Gingerol inhibits metastasis of MDA-MB-231 human breast cancer cells." **19**(5): 313-319.
29. Lee, S. H., M. Cekanova and S. J. J. M. C. P. i. c. w. t. U. o. T. M. A. C. C. Baek (2008). "Multiple mechanisms are involved in 6-gingerol-induced cell growth arrest and apoptosis in human colorectal cancer cells." **47**(3): 197-208.
30. Lee, T.-Y., K.-C. Lee, S.-Y. Chen, H.-H. J. B. Chang and b. r. communications (2009). "6-Gingerol inhibits ROS and iNOS through the suppression of PKC-α and NF-κB pathways in lipopolysaccharide-stimulated mouse macrophages." **382**(1): 134-139.
31. Lin, L.-I., Y.-F. Ke, Y.-C. Ko and J.-K. J. O. Lin (1998). "Curcumin inhibits SK-Hep-1 hepatocellular carcinoma cell invasion in vitro and suppresses matrix metalloproteinase-9 secretion." **55**(4): 349-353.
32. Longtin, R. (2003). "The pomegranate: nature's power fruit?" CancerSpectrum Knowledge Environment **95**(5): 346-348.
33. Mehta, K., P. Pantazis, T. McQueen and B. B. J. A.-c. d. Aggarwal (1997). "Antiproliferative effect of curcumin (diferuloylmethane) against human breast tumor cell lines." **8**(5): 470-481.
34. Mitra, A., J. Chakrabarti, A. Banerji, A. Chatterjee, B. J. J. o. E. P. Das, Toxicology and Oncology (2006). "Curcumin, a potential inhibitor of MMP-2 in human laryngeal squamous carcinoma cells HEp2." **25**(4).
35. Oyagbemi, A. A., A. B. Saba and O. I. J. B. Azeez (2010). "Molecular targets of [6]-gingerol: Its potential roles in cancer chemoprevention." **36**(3): 169-178.
36. Remondelli, P. and M. J. F. i. m. n. Renna (2017). "The endoplasmic reticulum unfolded protein response in neurodegenerative disorders and its potential therapeutic significance." **10**: 187.
37. Sang, S., J. Hong, H. Wu, J. Liu, C. S. Yang, M.-H. Pan, V. Badmaev, C.-T. J. J. o. a. Ho and f. chemistry (2009). "Increased growth inhibitory effects on human cancer cells and anti-inflammatory potency of shogaols from Zingiber officinale relative to gingerols." **57**(22): 10645-10650.
38. Schwertheim, S., F. Wein, K. Lennartz, K. Worm, K. W. Schmid, S.-Y. J. J. o. c. r. Sheu-Grabellus and c. oncology (2017). "Curcumin induces G2/M arrest, apoptosis, NF-κB inhibition, and expression of differentiation genes in thyroid carcinoma cells." **143**(7): 1143-1154.
39. Seo, D.-W., H. Li, L. Guedez, P. T. Wingfield, T. Diaz, R. Salloum, B.-y. Wei and W. G. J. C. Stetler-Stevenson (2003). "TIMP-2 mediated inhibition of angiogenesis: an MMP-independent mechanism." **114**(2): 171-180.
40. Shakibaei, M., T. John, G. Schulze-Tanzil, I. Lehmann and A. J. B. p. Mobasheri (2007). "Suppression of NF-κB activation by curcumin leads to inhibition of expression of cyclo-oxygenase-2 and matrix metalloproteinase-9 in human articular chondrocytes: implications for the treatment of osteoarthritis." **73**(9): 1434-1445.

Received 10 January 2024; Accepted 24 January 2024.

41. Sharma, P., S. McClees and F. Afaq (2017). "Pomegranate for prevention and treatment of cancer: an update." Molecules **22**(1): 177.
42. Shukla, Y., M. J. F. Singh and c. toxicology (2007). "Cancer preventive properties of ginger: a brief review." **45**(5): 683-690.
43. Su, C.-C., G.-W. Chen, J.-G. Lin, L.-T. WU and J.-G. J. A. r. Chung (2006). "Curcumin inhibits cell migration of human colon cancer colo 205 cells through the inhibition of nuclear factor kappa B/p65 and down-regulates cyclooxygenase-2 and matrix metalloproteinase-2 expressions." **26**(2A): 1281-1288.
44. Swarnakar, S., K. Ganguly, P. Kundu, A. Banerjee, P. Maity and A. V. J. J. o. B. C. Sharma (2005). "Curcumin regulates expression and activity of matrix metalloproteinases 9 and 2 during prevention and healing of indomethacin-induced gastric ulcer." **280**(10): 9409-9415.
45. Talmadge, J. E. and I. J. Fidler (2010). "AACR centennial series: the biology of cancer metastasis: historical perspective." Cancer research **70**(14): 5649-5669.
46. Weng, C. J., C. P. Chou, C. T. Ho, G. C. J. M. n. Yen and f. research (2012). "Molecular mechanism inhibiting human hepatocarcinoma cell invasion by 6-shogaol and 6-gingerol." **56**(8): 1304-1314.
47. Weng, C. J., C. F. Wu, H. W. Huang, C. T. Ho, G. C. J. M. n. Yen and f. research (2010). "Anti-invasion effects of 6-shogaol and 6-gingerol, two active components in ginger, on human hepatocarcinoma cells." **54**(11): 1618-1627.

i