Evaluation of the Anticancer Effect of *Xanthium Strumarium* Root Extract on Human Epithelial Ovarian Cancer Cells Using ^{1}H NMR-Based Metabolomics

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Abstract

Epithelial Ovarian cancer is the leading cause of cancer mortality among women all over the world. As chemotherapeutics has many side effects, researchers have focused on the potential use of medicinal plants as natural antitumor agents. *Xanthium strumarium* studied in this work as an herbal anticancer agent. This study aimed to evaluate the antitumor effect and metabolic alterations caused by the root extract of *X. strumarium* on human ovarian cancer cell line (A2780cp), using NMR-based metabolomics approaches. Cells were cultured and treated with different concentrations of the ethanolic plant extract. Antitumor activity determined by MTT assay and cell metabolites extracted for NMR spectroscopy. ^{1}H NMR spectroscopy was applied, and outliers were analyzed using multivariate statistical analysis techniques. The extract exhibited antitumor activity against ovarian cancer cells with an IC_{50} of 6 μg/ml after 48 hours of treatment. The most affected metabolic pathways in the experimental groups were limited to tyrosine metabolism, nucleotide metabolism, fatty acid biosynthesis, and glycerolipid metabolism. Our data showed that the ethanolic root extract of *X. strumarium* has antitumor activity on the ovarian cancer cells and can affect vital metabolic pathways. However, further studies required to validate this activity.

Keywords: *Xanthium strumarium*; Epithelial ovarian cancer; A2780cp cell line; Metabolomics; ^{1}H NMR.

Introduction

Cancer is a genetic disease caused by uncontrolled cell growth in the absence or disruption of cell cycle regulation, resulting in DNA damage [1]. Ovarian cancer is the fifth most common cancer in women and has the highest mortality rate among different types of cancers. Globally, the number of people get diagnosed with ovarian cancer is about 225,000, with 140,200 deaths per year [2]. During 2019, the American Cancer Society estimated that approximately 22,530 women diagnosed with ovarian cancer, among which about13,980 patients have died. About 90% of all malignant tumors are epithelial, originating from areas

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around the ovary, such as fallopian tubes and peritoneum [3]. The most effective chemotherapy drugs used to treat ovarian cancer are platinum analogs such as cisplatin and carboplatin. In most patients with advanced ovarian cancer, the disease recurs, associated with rising serum CA125 levels [4]. Treatment of ovarian cancer with conventional chemotherapy drugs can cause side effects. Natural compounds can prevent the development of invasive cancer with limited side effects. Hence, researchers have focused on the potential use of medicinal plants as natural anticancer agents [5].

X. Strumarium scrutinized in this investigation. The plant belongs to the Asteraceae (Compositae) family and commonly seen as a weed. It has widely grown in North America, Brazil, China, Malaysia, India, and Iran. Multiple studies have shown pharmacological effects of *X. strumarium*, including the anticancer impact on various types of cancers [6]. The inhibitory effect of leave extract of *X. strumarium* against cancer cell lines of A549 (lung), skov3 (ovary), SK-MEL-2 (Melanoma), XF498 (CNS) and HTC-15 (colon) reported. It was found that the seed extract of *X. strumarium* can inhibit the proliferation of HEPG2, Jurkat, L929, and A549 cancer cell lines. Fruit extract of *X. strumarium* had an antitumor effect against U937 and Jurkat cancer cell lines. The cytotoxic effect of root extract of *X. strumarium* on S180 and HEPG2 cancer cell lines have also demonstrated. A purified extract of aerial parts of *X. strumarium* possessed apparent antitumor activity on CT26 cancer cells. Several chemical compounds isolated from the roots of *X. strumarium*, among which coumarin was found only in the root of this plant [7]. Coumarin derivatives could inhibit the growth in human cancer cell lines such as HL-60 (leukemia), MCF7 (breast), H727 (lung), A549 (lung), and ACHN (renal). A coumarin derivative also had anticancer activity against ovarian cancer cell lines (OVCAR-3) [8]. Coumarin could be an active agent for treating side effects caused by radiotherapy [7]. Since the root of *the X. strumarium* plant enriched with coumarin, we would like to evaluate the antitumor potentiality of this extract.

Metabolomics is a powerful analytical tool to identify and detect qualitative changes in metabolites of biological systems and samples, resulting in recognizing the metabolites' biochemical pathways [9]. ^{1}H-NMR-based metabolomics is an emerging technology used to characterize cancer metabolism, with potential applications in clinical diagnosis or treatment of cancer [10].

The present study focuses on the antitumor effect of root extract of *X. strumarium* on the human ovarian cancer cell line(A2780cp) to find out if the obtained extract is effective in inhibiting the growth of epithelial ovarian cancer cells. Finally, altered metabolites and the most important metabolic pathways influenced by root extract were identified, using the human metabolome database (HMDB) and MetaboAnalyst software.

Materials and Methods

Plant Extract

Roots of *X. strumarium* collected from a region in Kermanshah, Iran, and authenticated by Central Herbarium of Tehran University under voucher No 48241. Collected plants were washed and dried at ambient temperature. 50 g of roots were powdered and extracted with 500 ml of 80% ethanol by the Soxhlet apparatus. The solvent removed by Rotary evaporator, the pure extract stored in a sterile container at 4°C for further use.

Cancer Cell Culture

A2780CP (human ovarian cancer cell line) obtained from the cell bank of Pasteur Institute of Iran. Cells were cultured in RPMI-1640 medium containing 10% FBS and penicillin/streptomycin (100X) as an antibiotic (Gibco). Cells were maintained and grown in a CO_2 incubator at 37°C until they reached above 85% confluency. A fixed number of cells (2×10^6 $_{/ml}$) used for all assays.

$$\% \, Viability = \frac{Live \; cell \; count}{total \; cell \; count} \times 100$$

Determination of Total Phenolic Content (TPC)

The concentration of total phenolics of *X. strumarium* root extract determined by the Folin-Ciocalteu test and external calibration with gallic acid as standard [11].

MTT assay

The effect of root extracts on cell growth measured by the MTT (3-4,5 dimethylthiazol-2,5 diphenyl tetrazolium bromide) assay. One mL cell suspension (2×10^6cells$_/$ml$_)$ was seeded into eight wells of a 96-well plate and incubated for 24hour. The monolayer of cells in each well was exposed to different concentrations (600, 60,30,15,12,7.5, 6 ,and 0.6 μg/ml) of plant root extract for 48 hours to determine the IC_{50}. MTT (20 μL) was added to each well and then incubated for 3 hours. After this, 180 μL of DMSO was added to each well and shake. The absorbance was read at 540 nm by Eliza Reader [1]. The Percentage of growth inhibition as calculated as follows:

$$\% \ Inhibition = 100 - \frac{Corrected \ mean \ Absorbance \ of \ sample}{Corrected \ mean \ Absorbance \ of \ control} \times 100$$

Cell extraction

Ten flasks (5 control samples and 5 test samples), each containing 80×10^6 cells/ml. were prepared. Test samples treated with 1920 µg/ml of root extract (according to the IC_{50} value), incubated, trypsinized, and centrifuged (1000 rpm, 4°C, 5min). PBS was added into each sample and centrifuged again. 0.5 ml of methanol and 1 ml of cold chloroform added to every 10^7 cells and vortexed. An equal volume of chloroform: double distilled water was added to the samples, sonicated (10 minutes), and centrifuged (4000rpm, 4°C, 15min), which resulted in the formation of 2 layers. The upper hydrophilic and the lower lipophilic layers transferred to clean tubes and lyophilized to obtain the NMR spectrum [12].

^{1}H NMR spectroscopy

Lyophilized powder of hydrophilic samples (n=10) resuspended in 700 µl of 100mM phosphate buffer (pH 7.0) prepared in D_2O and containing one mM TSP as an internal reference and two mM imidazole as pH indicator. Also, lipophilic samples (n=10) resuspended in 700 µl of deuterated chloroform. All samples were centrifuged at 10000 rpm for 10 minutes at 4°C. 500 µl of the extracted samples were transferred to NMR probes, analyzed on a Bruker AV-500 NMR spectrometer with field gradient operating at 500.13 MHz for proton observation at 298K. One-dimensional 1HNMR spectra recorded using a 10-µs pulse, 0.1 s mixing time, 3.0 s relaxation delay, 6009.6 Hz spectral width, and 3000 transients with standard 1D NOESY pulse sequence to suppress the residual water peak [13].

Data Analysis

NMR spectra preprocessed using ProMetab software (V.3.3) in MATLAB (v.7.8.0.347) environment. In the following, each spectrum was aligned and binned at 0.005 ppm. The water peak (4.7 ppm) removed. Multivariate analysis of data performed by using PLS-DA. The discrimination chemical shifts reported as score plot, loading plot, and VIP scores. To identify outliers, projected chemical shifts taken to the HMDB database, and matched metabolites identified. MetaboAnalyst (V.4.0), is a set of online tools for metabolomic data analysis, and interpretation was used to work out the most important metabolic pathways altered in this research [14].

Results

Total polyphenol content

The total phenolic content of the root extract of *X. strumarium* was estimated to be 6000 µg/ml.

Proliferation Assay

The root extract also exhibited anticancer activity against A2780cp cells with an IC_{50} of 6 µg/ml.
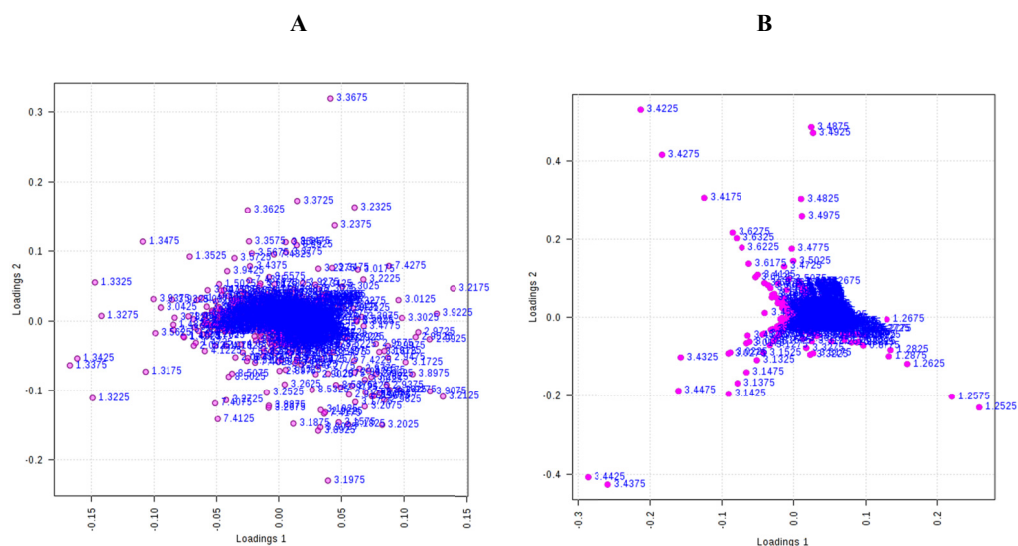
A   **B**

Figure 1. PLS-DA loading plot for hydrophilic (A) and lipophilic (B) groups of A2780cp ovarian cancer cells.

Effect of root extract of *X. Strumarium* on metabolome profile

1D NMR assay showed that many metabolic pathways influenced by the root extract of *X. strumarium*. In the current study PLS-DA, discrimination analysis resulted in the classification of data, as shown in loading plots between the selected components (Figure 1). PLS-DA identified important features as variable importance in projection (VIP) scores (Figure 2).

The spots inside the graphs represent the metabolite's chemical shift bins. The loading 2 –axis denotes the correlation of the bins towards the predictive variation. The loading 1-axis signifies the magnitude of the spectral bins .The mass spots are related to the common metabolites in both cell groups, and on the contrary, separate spots are altered metabolites that expected in the current study.

A VIP score is a measure of a variable's importance (chemical shifts) in the PLS-DA model. The Y-axis indicates the VIP scores related to each variable on the X-axis. The colored boxes on the right show the relative concentrations of the corresponding metabolite in the test and control cell groups.

Pathway analysis

HMDB database used to identify metabolites related to spectral bins. Also, metabolic pathways corresponding to these separated outliers determined by the MetaboAnalyst database. Tables 1 and 2 show discriminate metabolic pathways analysis.

Discussion

Excessive proliferation of cancer cells and their higher needs for energy and specific nutrients causes remarkable metabolic variations compared to healthy cells [15]. Recent years have witnessed the expanded use of metabolomics approaches to study cancer treatment [9]. The availability of natural products with higher effectiveness and lower side effects is desired [5]. In this study, a root extract of X. *strumarium* exhibited productive anticancer activity against epithelial ovarian cancer A2780CP cell line.

Many tumor types, including epithelial ovarian cancer, were shown to reprogram their metabolism during progression, switching from oxidative phosphorylation to glycolysis, a phenomenon known as the Warburg effect [16]. Also, epithelial ovarian cancer shows alterations in the central metabolic pathways, including glycolysis, the tricarboxylic acid cycle, amino-acid, and lipid metabolism. These metabolic pathways are interconnected, and their dysregulation contributes to cancer onset and progression [15].
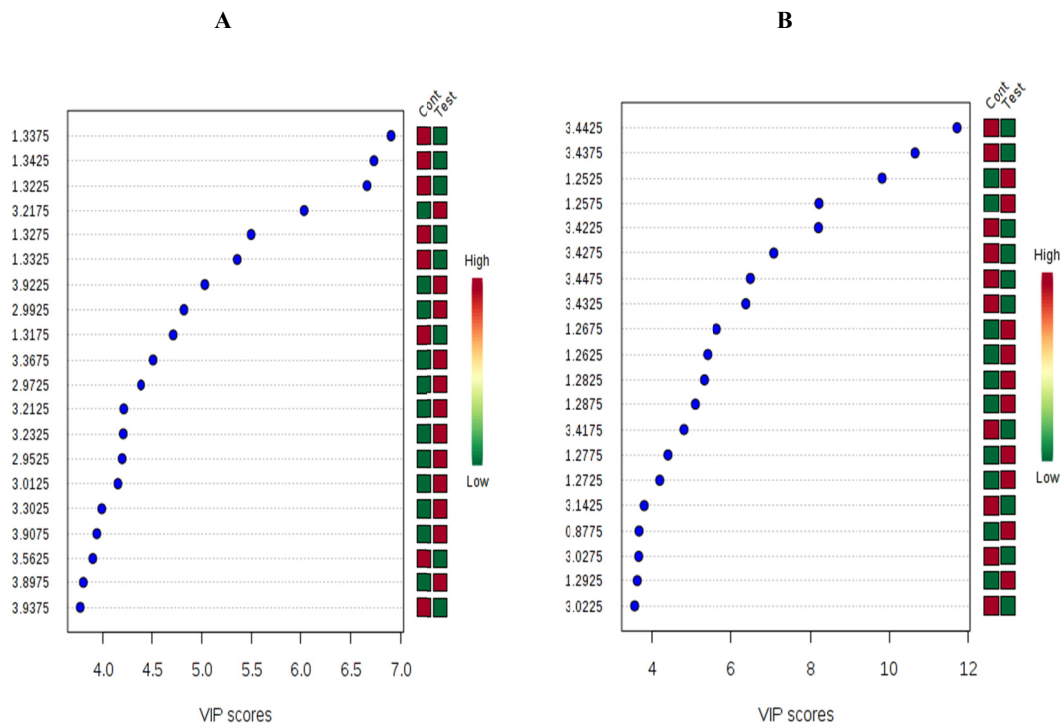

Figure 2. VIP scores for hydrophilic (A) and lipophilic (B) groups of A2780cp ovarian cancer cells.

Table 1. Altered hydrophilic metabolites and the most important metabolic pathways in A2780Cp ovarian cancer cells were influenced by the root extract of *X. strumarium*

Metabolic pathway	Metabolites	Total	Hits	Raw P
Tyrosine metabolism	L-tyrosine	76	7	0.0002
	Iodotyrosine			
	L-dopa			
	3,5-Diiodo-L-tyrosine			
	3,4-Dihydroxyhydrocinnamic acid			
	Dopamine			
	Normetanephrine			
Amino sugar and nucleotide sugar metabolism	Glucose1-phosphate	88	6	0.0032
	Glucose 6-phosphate			
	D-mannose			
	D-xylose			
	Glucosamine 6-phosphate			
	Fructose 6-phosphate			
Pentose and glucuronate interconversions	L-Arabitol	53	4	0.0116
	Glucose 1-phosphate			
	D-xylose			
	Fructose 6-phosphate			
Galactose metabolism	D-mannose	41	3	0.0312
	Glucose1-phosphate			
	Glucose 6-phosphate			
Starch and sucrose metabolism	Glucose1-phosphate	50	3	0.0517
	Glucose 6-phosphate			
	D-xylose			

Total, the total number of compounds in the pathway; Hits, the actual matched number from the user uploaded data; Raw p, the original p-value calculated from the enrichment analysis, using MetaboAnalyst database

Table 2. Altered lipophilic metabolites and the most important metabolic pathways in A2780Cp ovarian cancer cells were influenced by the root extract of *X. strumarium*

Metabolic pathway	Metabolites	Total	Hits	Raw p
Fatty acid biosynthesis	Capric acid	49	3	0.0238
	Oleic acid			
	Stearic acid			
Glycerolipid metabolism	Propylene glycol	32	2	0.062
	Triacylglycerol			
Biotin metabolism	Pimelic acid	11	1	0.1331
Citrate cycle (TCA cycle)	Cis-aconitic acid	20	1	0.2291

Total, the total number of compounds in the pathway; Hits, the actual matched number from the user uploaded data; Raw p, the original p-value calculated from the enrichment analysis, using MetaboAnalyst database

In the current study, many metabolites were altered after treated with the *X. strumarium* root extract. Mapping these different metabolites to their biochemical pathways through HMDB and KEGG search revealed that the most affected metabolic pathways involved in cell growth inhibition were limited to tyrosine metabolism, nucleotide metabolism, fatty acid biosynthesis, and glycerolipid metabolism.

Tyrosine, synthesized from phenylalanine, is metabolized by four enzymes. It catalyzed to fumarate and acetyl coenzyme A (acetyl-CoA). Fumarate is an intermediate in the tricarboxylic acid cycle in which ATP is produced [17].

Tyrosine and phenylalanine were higher in ovarian cancer cell lines compared to healthy cells. Similarly,

higher TCA cycle intermediates (including succinate, fumarate, and malate) have observed in tissue samples from ovarian carcinoma [18]. Increased levels of both branched-chain amino acids and aromatic amino acids are related to alterations in their transporter activity, L-type amino acid transporter 1 (LAT1), which is an essential molecule in the nutrition, proliferation, and migration of ovarian cancer cells [19].

In this research, several metabolites altered in tyrosine metabolism. Hence, a comparison of our results with previous reports showed that the root extract could inhibit the growth of ovarian cancer cells, which may through disrupting the TCA cycle and inhibiting LAT1.

Nucleotides are necessary for a variety of cellular processes. It has well-characterized that imbalances in

nucleotide levels lead to a variety of human diseases, including cancer. Tumor cells have a higher need for dNTPs [20]. The synthesis of nucleotides begins with the formation of ribose-5-phosphate from glucose. Along with glutamine, which donates the necessary nitrogen, several modifications lead to the formation of nucleotides. A change in RRM1, RRM2, or p53R2 gene expression can lead to dNTP pool imbalances. Ultimately, this imbalance leads to replication stress and impaired DNA damage repair, which consequently increases DNA damage, genomic instability, and mutations. These ultimately contribute to cell transformation and cancer. Since nucleotide metabolism plays a role in transformation and tumor progression, inhibition of this pathway has long been considered a therapeutic strategy for cancer [21].

In this study, we observed that Glucose1-phosphate, Glucose 6-phosphate, D-mannose, D-xylose, Glucosamine 6-phosphate, and Fructose 6-phosphate altered in nucleotide metabolism.

Alteration of glucose 1-phosphate and glucose 6-phosphate can disrupt the glycolysis pathway. D-xylose linked to pentose and glucuronate interconversion pathway, so the change of this metabolite can disrupt this pathway leading to reduce energy in cancer cells. D-mannose directly linked to fructose and mannose metabolism, so alteration of this metabolite can disrupt fructose and mannose metabolism, also leading to inhibition of cancer cell's energy. Altered fructose 6-phosphate is directly linking to the glycolysis pathway, can disrupt this pathway. According to the Warburg effect theory, cancer cells use glycolysis cycle as the most critical energy sources. Hence, it seems that fructose 6-phosphate can be a potential therapeutic target in ovarian cancer. A comparison of our findings with previous findings showed that the root extract could inhibit the growth of cancer cells, probably by disrupting DNA synthesis and reducing cellular energy.

Variation of lipid metabolism was detected in EOC patients at both the early and late stages of the disease and in patients with recurrent disease [22]. Fatty acid (FA) synthesis significantly depends on glucose through the production of acetyl-CoA, a central metabolic precursor. In tumor cells, most FAs are synthesized *de novo* by fatty acid synthase (FASN) to arrange the intensive bioenergetics and structural changes. Indeed, FASN has defined as a marker of cell proliferation and a drug target in oncology [23]. Increasing palmitic acid due to amplified expression of fatty acid synthase has a very close relationship with cirrhotic tissue in ovarian cancer and suppression of this enzyme activity leading to induce apoptosis and cancer cell death [24]. Another investigation has proposed that expression of fatty acid

synthase gene significantly increases in a wide range of tumors (colon, prostate, breast, and ovary) by reasons of the insensitivity of tumor cells to regulatory messages and the higher tendency of cancer cells to *de novo* lipogenesis pathway [25].

The involvement of stearic acid in cancer has reported, and such as induction of apoptosis that prevents the development of cancer cell formation; on the other hand, it has a negative role in DNA damage that promotes cell transformation and, in turn, tumor genesis[24]. It is also observed that a reduction in the ratio of stearic acid and oleic acid might serve as an indication of malignancy [26]. High level of oleic acid and low level of stearic acid reported in several cancer types such as breast, prostate, liver, pancreas, colon, and lung [27]. Another study also showed that oleic acid blocked the activity of fatty acid synthase and accumulated malonyl CoA, which led to the suppression of HER2 oncogene expression [28].

In the present study, capric acid, oleic acid, and stearic acid altered in Fatty acid biosynthesis. Thus, it seems that oleic acid and stearic acid can be potential therapeutic targets in ovarian cancer. A comparison of our results with previous investigations showed that the extract could inhibit the growth of cancer cells, probably through inhibition of FASN activity.

Variations in the lipid metabolism, particularly serum triglycerides, have been observed in breast and ovarian cancer patients, representing evidence for a positive association between triglycerides and the risk of developing these cancers [29]. It revealed a positive correlation between serum TG levels with endometrial and colon cancer risk [30]. A few cohort studies have investigated the high serum triglyceride concentrations as a part of the metabolic syndrome about the risk of colon, breast, and cervix cancers. A cohort study among Icelanders revealed the associations between serum TG and thyroid cancer in men and with cervix, endometrial, and bladder cancer in women [31]. Positive associations also found with gynecological cancers among women.

In this study, propylene glycol and triacylglycerol metabolites altered in glycerolipid metabolism. Hence, it seems that triacylglycerol can be a potential therapeutic target in ovarian cancer treatment. A comparison of our findings with earlier reports showed that the root extract could inhibit cancer cells' growth, probably by altering of triacylglycerol levels [32].

Conclusion

In this preliminary study, an ethanolic root extract of *X. strumarium* exhibits antitumor activity even in low doses. It affects vital metabolic pathways of epithelial ovarian cancer cells, particularly lipid metabolism.

However, to confirm these activities, further research is underway to validate these findings along with the potential fractions of the root extract of this plant.

Acknowledgment

References

1. Vaishnav K., George L.B. & Highland H. N. Antitumour activity of Xanthium strumarium L. on human cervical cancer HeLa cells. *J. Cancer Tumor Int.* 1–13 (2015).
2. Siegel R. L., Miller K. D. & Jemal A. Cancer statistics, 2016. *CA. Cancer J. Clin.* **66**: 7–30 (2016).
3. Smith R. A*., Andrews KS., Brooks D., Fedewa SA., Manassaram-Baptiste D., Saslow D., Wender RC.* Cancer screening in the United States, 2019: A review of current American Cancer Society guidelines and current issues in cancer screening. *CA. Cancer J. Clin.* **69**: 184–210 (2019).
4. Zhong L. X., Wu M.L., Li H., Liu J. & Lin L.Z. Efficacy and safety of intraperitoneally administered resveratrol against rat orthotopic ovarian cancers. *Cancer Manag. Res.* **11**: 6113 (2019).
5. Lichota A. & Gwozdzinski K. Anticancer activity of natural compounds from plant and marine environment. *Int. J. Mol. Sci.* **19**: 3533 (2018).
6. Al-Mekhlafi F. A., Abutaha N., Mashaly AMA., Nasr FA., Ibrahim KE., Wadaan MA. Biological activity of Xanthium strumarium seed extracts on different cancer cell lines and Aedes caspius, Culex pipiens (Diptera: Culicidae). *Saudi J. Biol. Sci.* **24:** 817–821 (2017).
7. Fan W., Fan L., Peng C., Zhang Q., Wang L., Li L., Wang J., Zhang D., Peng W., Wu C. Traditional Uses, Botany, Phytochemistry, Pharmacology, Pharmacokinetics and Toxicology of Xanthium strumarium L.: A Review. *Molecules* **24**: 359 (2019).
8. Klenkar J. & Molnar M. Natural and synthetic coumarins as potential anticancer agents. *J. Chem. Pharm. Res* **7**: 1223–1238 (2015).
9. Pinu F. R., Goldansaz S. A. & Jaine J. Translational Metabolomics: Current Challenges and Future Opportunities. *Metabolites* **9**.108 (2019).
10. Emwas A.-H., Roy R., McKay RT., Tenori L., Saccenti E., Gowda GAN., Raftery D., Alahmari F., Jaremko L., Jaremko M., Wishart DS. NMR spectroscopy for metabolomics research. *Metabolites* **9**: 123 (2019).
11. Scherer R. & Godoy H. T. Effects of extraction methods of phenolic compounds from Xanthium strumarium L. and their antioxidant activity. *Rev. Bras. Plantas Med.* **16**: 41–46 (2014).
12. Gottschalk M., Ivanova G., Collins DM., Eustace A., OConnor R., Brougham DF. Metabolomic studies of human lung carcinoma cell lines using in vitro 1H NMR of whole cells and cellular extracts. *NMR Biomed. An Int. J. Devoted to Dev. Appl. Magn. Reson. vivo* **21**: 809–819 (2008).
13. Sheedy J. R. Metabolite analysis of biological fluids and tissues by proton nuclear magnetic resonance spectroscopy. In *Metabolomics Tools for Natural Product Discovery* 81–97 (Springer, 2013).
14. Smolinska A., Blanchet L., Buydens L. M. C. & Wijmenga S. S. NMR and pattern recognition methods in metabolomics: from data acquisition to biomarker discovery: a review. *Anal. Chim. Acta* **750**: 82–97 (2012).
15. Sreedhar A. & Zhao Y. Dysregulated metabolic enzymes and metabolic reprogramming in cancer cells. *Biomed. Reports* **8**: 3–10 (2018).
16. Warburg O. On the origin of cancer cells. *Science (80-.).* **123**: 309–314 (1956).
17. Shan C., Lu Z., Sheng H., Fan J., Qi Q., Liu S., Zhang S. 4-hydroxyphenylpyruvate dioxygenase promotes lung cancer growth via pentose phosphate pathway (PPP) flux mediated by LKB1-AMPK/HDAC10/G6PD axis. *Cell Death Dis.* **10**: 525 (2019).
18. Halama A., Guerrouahen BS., Pasquier J., Diboun I., Karoly ED., Suhre K., Rafii A. Metabolic signatures differentiate ovarian from colon cancer cell lines. *J. Transl. Med.* **13**: 223 (2015).
19. Kaji M., Kabir-Salmani M., Anzai N., Jin CJ., Akimoto Y., Horita A., Sakamoto A., Kanai Y., Sakurai H., Iwashita M. Properties of L-type amino acid transporter 1 in epidermal ovarian cancer. *Int. J. Gynecol. Cancer* **20**: 329–336 (2010).
20. Aird K. M. & Zhang R. Nucleotide metabolism, oncogene-induced senescence, and cancer. *Cancer Lett.* **356**: 204–210 (2015).
21. Rizzo A., Napoli A., Roggiani F., Tomassetti A., Bagnoli M., Mezzanzanica D. One-carbon metabolism: biological players in epithelial ovarian cancer. *Int. J. Mol. Sci.* **19**: 2092 (2018).
22. Garg G., Yilmaz A., Kumar P., Turkoglu O., Mutch DG., Powell MA., Rosen B., Bahado-Singh RO., Graham SF. Targeted metabolomic profiling of low and high grade serous epithelial ovarian cancer tissues: a pilot study. *Metabolomics* **14**: 154 (2018).
23. Kisková T. & Kassayová M. Resveratrol Action on Lipid Metabolism in Cancer. *Int. J. Mol. Sci.* **20**: 2704 (2019).
24. Yu X., Ren X., Liang X. & TangY. Roles of fatty acid metabolism in tumourigenesis: Beyond providing nutrition. *Mol. Med. Rep.* **18**: 5307–5316 (2018).
25. UedaS M*., Yap KL., Davidson B., Tian Y., Murthy V., Wang TL., Visvanathan K., Kuhajda FP., Bristow RE., Zhang H., Shih IeM.* Expression of fatty acid synthase depends on NAC1 and is associated with recurrent ovarian serous carcinomas. *J. Oncol.* **2010**. (2010).
26. Ricciardi M. R., Mirabilii S., Allegretti M., Licchetta R., Calarco A., Torrisi MR, Foà R., Nicolai R., Peluso G., Tafuri A. Targeting the leukemia cell metabolism by the CPT1a inhibition: functional preclinical effects in leukemias. *Blood* **126**:1925–1929 (2015).
27. Amézaga J., Arranz S., Urruticoechea A., Ugartemendia G., Larraioz A., Louka M., Uriarte M., Ferreri C. Tueros Altered Red Blood Cell Membrane Fatty Acid Profile in Cancer Patients. *Nutrients* **10**: 1853 (2018).
28. Flavin R., Peluso S., Nguyen P. L. & Loda M. Fatty acid synthase as a potential therapeutic target in cancer. *Futur. Oncol.* **6**: 551–562 (2010).

29. Zeleznik O. A., Eliassen H., Kraft P., Poole E.M., Rosner B., Jeanfavre S., Deik A., Bullock K., Hitchcock D., Avila-Pancheco J., Clish C.B., Tworoger S.S. A prospective analysis of circulating plasma metabolomics and ovarian cancer risk. *bioRxiv* 654962 (2019).

30. Hirasawa A., Makita K., Akahane T., Yokota M., Yamagami W., Banno K., Susumu N., and Aoki D.Hypertriglyceridemia is frequent in endometrial cancer survivors. *Jpn. J. Clin. Oncol.* **43**: 1087–1092 (2013).

31. Tulinius H., Sigfússon N., Sigvaldason H., Bjarnadóttir K. & Tryggvadottir L. Risk factors for malignant diseases: a cohort study on a population of 22,946 Icelanders. *Cancer Epidemiol. Prev. Biomarkers* **6**: 863–873 (1997).

32. Long J., Zhang C.J., Zhu N., Du K.,Yin Y.F., Tan X., Lio D.F., and Qin L. Lipid metabolism and carcinogenesis, cancer development. *Am. J. Cancer Res.* **8**: 778 (2018).