2)

عنوان المشروع باللغة العربية - Title of the proposed project in Arabic	ميكانيكيات الخاصية المضادة للسرطان للأنيثول:استهداف المايكرو RNA المنخفض التنظيم في سرطان الثدي
Title of the proposed project in English	Mechanisms of anticancer propriety of Anethole: Targeting the profile of MicroRNA down-regulated in Breast cancer
المشرف الرئيس - PI	**عبير عبد القادر الغنوشي**
التخصص الدقيق للمشرف الرئيس - Specialty of PI	البروتينات و الكيمياء الحيوية الكلينيكية
المشرف المساعد - Co-PI	**عبد الحبيب السملالي**
المدة المتوقعة لإنجاز البحث منذ الحصول على موافقة عمادة الدراسات العليا (بالشهور) - Expected time in month to finish	12

Abstract of the proposal (No more than 200 words)	Adjuvant breast cancer therapy is based on the use of anti-estrogen but after a period of time develops phenomena of resistance. New generation of therapies for advanced breast cancer is still a cause for researchers' concern. In the past decades, a lot of research has been conducted to discover the natural compounds with potential antitumor activity. Anethole is an aromatic compound that occurs widely in nature as a major and active component of anise, foeniculum vulgare, licorice, and star anise (Chainy et al., 2000). It is widely used for foods flavoring and beverages due to its pleasant spicy aroma (Rather et al., 2016). Recently, Anethole has been identified as a potential phytochemical with anti-cancer properties which could be developed into clinical treatments for cancer therapy (Villarini et al., 2014, Chen et al., 2009). It possesses many beneficial properties, including antioxidant, anti-inflammatory, antibacterial, and anticancer. Anethole was found to inhibit estrogen-sensitive and estrogen-independent breast cancer cell lines' growth and induced apoptosis. However, the mechanisms that mediate the anti-cancer effects of Anethole remain largely unknown. MicroRNAs (miRs), a new class of small RNAs, were found to be able to influence the tumor progression by modulating both mRNA stability and the ability of mRNA to be translated into protein. This project will focus on the mechanism of action of Anethole toward miRNAs. miR PCR-array will be used to compare the miRNAs in the breast cancer cells with and without Anethole. Moreover, the association between Anethole and the breast cancer progression will be investigated.
Hypothesis of the proposal	Because of their ability to influence the tumor progression, miRNAs might take part in the effect of Anethole on breast cancer cells progression by using microRNA (miR) array analysis.

Specific objectives	This project aims to investigate: 1- The effect of Anethole on microRNA expression by qPCR- Array 2- The correlation between the down regulation of micoRNA expression by Anethole and predictive factor of breast cancer tissue 3- The effect of the overexpression of miRs selected on the Anethole effect (breast cancer cells proliferation and migration)
Methodology & Major Techniques to be used	1- Objective1 : Effect of Anethole on microRNA expression by qPCR- Array a) Breast cancer Cell culture The MCF-7 and MDA322 cells will be purchased from ATCC company and maintained in Dulbecco's Modified Eagle's Medium (Thermo Fisher Scientific, Waltham, MA, USA) containing 10% heat-inactivated fetal bovine serum albumin (Invitrogen, MA, USA) in 95% humidified incubator with 5% CO2 at 37C with penicillin–streptomycin (10,000 U/mL). b) MicroRNA extraction, Reverse transcription and detection of differentially expressed microRNA by quantitative real-time PCR array Total microRNA will be isolated from the breast cancer cells using mirVana Tm miRNA isolation kit (Ambion , USA) according to the manufacturer's instructions. To quantify the mirRNA concentration and purity, we will use the Agilent 2100 Bio analyzer system and Agilent Small RNA analysis kit according to the manufacturer's instructions (Agilent Technologies, Santa Clara, CA, USA) and a NanoDrop8000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). cDNA will be synthetized from 1 µg of each microRNA sample's with a high-capacity cDNA reverse transcription kit from Applied Biosystems (Foster City, CA, USA). The PCR Array will be performed as previously described by Semlali et al. using miScript miRNA PCR Array Human Breast Cancer from Qiagen (Qiagen Company, USA) following the

	manufacturer's instructions. The number of copies of the micro RNA will be measured using the Applied Biosystems 7500 Fast real-time PCR detection system. The specificity of each primer pair will be verified by the presence of a single melting temperature peak, and the data will be analyzed using the ΔΔCt method and fold changes between control and anethole stimulated samples were calculated. Micro RNA expression will be selected if a fold change above 1.5. 2- Objective 2: Correlation between the down regulation of micoRNA expression by Anethole and predictive factor of breast cancer tissue a) Patients and specimens The present study will be a retrospective analysis which includes 20 patients with primary breast cancer newly diagnosed from July 2015 to July 2017 at King Khaled Hospital. This retrospective analysis was approved by the Research Ethics Committee of King Khaled Hospital. All patients voluntarily joined this study and signed informed consents. The breast cancer blots tissues collected by for each patient to analyze the miRs levels using the qRT-PCR method. b) qRT-PCR The mirVana Tm miRNA isolation kit will be used to purify total miRNA from breast cancer and normal breast tissue samples. The complementary DNA was synthesized reverse Transcriptase Kit (Thermo Fisher Scientific). The quantitative analysis of the miRNA selected by PCR Array level will be performed using qRT-PCR, and GAPDH will be used as an endogenous control. qRT-PCR will be conducted using the Applied Biosystems 7500 Fast real-time PCR detection system. The fold changes will be calculated through the relative quantification using $2-\Delta\Delta Ct$. All the reactions will be performed in a 20 µL reaction volume in triplicate. c) In situ hybridization analysis Breast cancer tissues for in situ hybridization (ISH) will be fixed in 4% paraformaldehyde solution, dehydrated in a graded series of ethanol baths, and embedded in paraffin. Then, the tissue slides will be deparaffinized and digested with proteinase K for 30 minutes. After that, the slides will

	prehybridized in a hybridization solution at 57°C for 2 hours. Tissues will be hybridized overnight in the presence of 10 ng 3′-5′biotin- labeled miRNA selected (Exiqon) probes at 63°C. Slides will be washed twice stringently and an immunological reaction will be carried out by using the rabbit antibody against biotin and alkaline phosphatase, according to the manufacturer's recommendation. Each side will be assigned a score for intensity and staining positive pattern. Objective 3: Effect of the overexpression of miRs selected on the Anethole effect (Breast cancer cells proliferation and migration) a) Proliferation by MTT assay The Check2 plasmid (Promega Corporation, Fitchburg, WI, USA) will be used for the over-expression of the miRNA selected. The proliferation will be evaluated by MTT assay after stimulation by anethole for 12h, 24h and 48h according to Semlali et al. (2011). Statistical analysis The data will be expressed as mean ± standard deviation of at least three independent experiments. One-way analysis of variance will be used to test the differences between the mean values. All P-values will be two sided, and a value of 0.05 will be considered to be statistically significant. All statistical calculations will be performed using Statistical Package for the Social Sciences software (version 18.0; SPSS Inc., Chicago, IL, USA).
Availability of Samples	Yes
Availability of Chemicals	Yes

Availability of Instruments	Yes
Ethical Approval	Ethical approval is available
Recent References	RATHER, M. A., DAR, B., SOFI, S.N., BHAT, B. A., QURISHI. M.A. 2016. Foeniculum vulgare: A comprehensive review of its traditional use, phytochemistry, pharmacology, and safety. Arabian Journal of Chemistry, 9, S1574-S1583. SEMLALI, A., CHAKIR, J. & ROUABHIA, M. 2011. Effects of whole cigarette smoke on human gingival fibroblast adhesion, growth, and migration. J Toxicol Environ Health A, 74, 848-62. SIEGEL, R. L., MILLER, K. D. & JEMAL, A. 2017. Cancer Statistics, 2017. CA Cancer J Clin, 67, 7-30.