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Essential oil from *Cymbopogon flexuosus* as the potential inhibitor for HSP90

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ABSTRACT

The essential oil of *Cymbopogon flexuosus* or lemongrass oil (LO) is reported to have antibacterial, antifungal and anticancerous effects. HSP90 is one of the major chaperones responsible for the proper folding of cancer proteins. In this paper we show that the essential oil of *C. flexuosus* significantly suppresses the HSP90 gene expression. The cytotoxicity of the compounds was tested by MTT assay and the gene expression studies were carried out using HEK-293 and MCF-7 cells. Also we tested the efficacy of the major component of this essential oil viz. citral and geraniol in inhibiting the HSP90 expression. The oil was found to be more cytotoxic to MCF-7 cells with different IC_{50} values for the oil (69.33 µg/mL), citral (140.7 µg/mL) and geraniol (117 µg/mL). The fold change of expression was calculated by RT-qPCR using ΔΔCt ($2^{-\Delta\Delta Ct}$) method and it was 0.1 and 0.03 in MCF-7 cells at 80 µg/mL and 160 µg/mL of LO. Western blot results showed suppression of HSP90 protein expression and HSP90 – ATPase activity inhibition was also observed using LO. This study shows the anticancer mechanism exhibited by the essential oil of *C. flexuosus* is by the inhibition of the important chaperone protein HSP90.

1. Introduction

Breast cancer was found to be the second highest cause of death in women in USA and as of March 2017 statistics, more than 3.1 million women with a history of breast cancer [1]. Breast cancer is classified into subtypes based on the receptors expressed on the tumour cells viz. estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth receptor (HER 2) [2]. The triple negative subtype had the minimum survival. MCF-7 cells are extensively used human breast adenocarcinoma cells with the useful characteristics similar to mammary epithelium, having the capability to process estrogen (ER positive) [3]. The estrogen induces the proliferation of MCF-7 and the therapy for breast cancer targets mainly the blocking of estrogen action [4]. The anti-estrogens are used as therapeutics against the ER + ve tumours have the inhibitory effects on MCF-7 [5]. Estrogen binds to the DNA and modulates the gene expression of ER [6]. Selective estrogen receptor modulators (SERMS) i.e. tamoxifen compete with estrogen for ER binding and thus the antiestrogen therapy is one of the major therapies in ER positive breast cancer [7].

The treatment with anti-estrogen drugs like tamoxifen is the most preferred choice for the ER positive breast cancers. The resistance developed against the antiestrogen therapy is becoming a major problem in treating the estrogen dependent breast cancers. The de novo resistance is the complete absence of the estrogen receptor function or it may be the acquired resistance by the unresponsiveness of the hormone receptors to the anti-estrogens [8]. Different compositions consisting of the admixture of different essential oils and pharmaceutically acceptable carrier has been reported to be useful in the treatment of breast cancer [9]. The anti-proliferative and anti-estrogenic properties of the essential oils could be useful as alternative therapy in treating estrogen dependent cancers. Though there are monoterpenoids like linalool, d-limonene and α-terpineol have been studied for potential treatment for breast cancer, there are no reports about their anti-estrogenic mechanism.

The essential oil of *C. flexuosus* is reported to inhibit the proliferation of various cancer cells [10]. A component of LO i.e. citral, an important monoterpene and a flavouring agent is reported to inhibit the proliferation of human and murine cancer cell lines A2780, ECC-1,

Abbreviations: DEPC, diethyl pyrocarbonate; DMEM, Dulbecco's modified Eagle's medium; DMSO, dimethyl sulfoxide; ER, estrogen receptor; FBS, fetal bovine serum; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GC–MS, gas chromatography–mass spectrometry; HER 2, human epidermal growth receptor 2; HPLC, high performance liquid chromatography; HSP90, heat shock protein 90; LO, lemongrass oil; MTT, 3-(4,5-dimethythiazol-. 2-yl)-2,5-diphenyl tetrazolium bromide; PR, progesterone receptor; RT-qPCR, reverse transcriptase quantitative polymerase chain reaction; SERMS, selective estrogen receptor modulators

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OVCAR-3, SKOV-3, ID8 and MOVCAR [11]. Citral is reported to induce benign and atypical prostate hyperplasia [12] and also the hormone sensitive tissue responsiveness was reported by its anti-estrogenic actions [13]. One of the precursors of citral i.e. geraniol was also found to induce gynacomastia in man following its use [14]. In another study both citral and geraniol, did not show estrogenic or antiestrogenic actions at lower concentrations. Also these compounds did not show any estrogenic response in overiectemized mice. However at higher concentrations both citral and geraniol showed anti-estrogenic activity in estrogen responsive human cell line Ishikawa var – I [15]. Citral is also reported to inhibit the proliferation and also inducing apoptosis in estrogen receptor positive MCF-7 cells [16].

There are other compounds isolated from the natural sources and used in folk remedy reported to induce apoptosis in MCF-7 cells. Farnesiferol-C which was isolated from *Ferula asafoetida* (a type of coumarins) is induces cell cycle arrest and apoptosis in MCF-7 cells [17]. Also the naturally occurring secondary metabolites viz. depsidones isolated from marine fungus also have shown to inhibit tumour cell proliferation in T47D breast tumour cells [18]. These studies show the anticancer potential of the compounds used in folk medicines.

Though the anticancer properties are reported for the lemongrass essential oil, we report the anticancer mechanism by the inhibition of the important chaperone involved in oncogenic pathways viz. HSP90 gene and protein. The present study gives an understanding of the effect of lemongrass oil and its constituents viz. citral and geraniol on gene and protein expression of HSP90 in MCF-7 cells in comparison with normal HEK-293 cells. Also here we study the functional inhibition of HSP90-ATPase activity using pure HSP90 protein. To separate the components of LO and evaluate the mechanism of binding of those components into the N-terminal or C-terminal domains of HSP90 to study the interactions would be the future perspective for this study

2. Materials and method

MCF-7 (HTB22) and HEK-293 (CRL1573) were obtained from ATCC. The essential oil of *C. flexuosus* (LO) was obtained from Perfect Herbals and Oils, Madhya Pradesh, India. Citral (95%) and geraniol (98%) were obtained from Sigma, India. The essential oil, citral and geraniol were analysed using the newly developed HPLC method in our laboratory and also by GC–MS [19]. The DMEM medium, FBS, penicillin G, streptomycin were obtained from Invitrogen, India. MTT powder was obtained from Sigma, India. The solution is filtered through a 0.2 μ m filter and stored at 2–8 °C for frequent use or frozen for extended periods. DMSO and DEPC water, trizol and isopropanol were obtained from Sigma, India. Tecan Plate reader was from Tecan, Switzerland. The chloroform and ethanol were obtained from Ranchem. PBS required for cell culture was obtained from Himedialabs, India. 1.5 and 2 mL vials were obtained from Eppendorf and 15 mL falcon tubes were obtained from Tarsons Ltd. The vials and tubes were washed with DEPC treated water, autoclaved and oven dried. Human HSP90 protein was obtained from Stressmarq Biosciences, Canada. ATP– Sodium salt was obtained from Sigma, India. Other reagents used in the HSP90 – ATPase inhibition experiement were of analytical grade.

The rabbit polyclonal antibody against HSP90 (sc-7947) was obtained from Santa-Cruz, Biotechnology, USA. The secondary anti-rabbit antibodies were from Invitrogen. The beta actin primary antibody (MA5-15739) and the anti-mouse secondary antibodies were also procured from Invitrogen. The SDS-PAGE and western blot analysis were done using the buffers from Invitrogen and ECL kit from Thermo Scientific-Pierce.

2.1. Cytotoxicity study

Cytotoxicity of LO and citral was tested on breast cancer cell lines MCF-7 using MTT assay. Cells were collected when they reach about 70–80% confluency. Cells were checked for viability using Trypan blue staining and centrifuged. The wells were seeded at 50,000 cells / well of MCF-7 in a 96 well plate and incubated for 24 h at 37 °C, 5% CO_2 incubator. The essential oil, citral and geraniol were added from 5 to 320 μg/mL concentration in DMEM without FBS and incubated for 24 h. After incubation with test samples, filtered MTT (5 mg/10 mL of MTT in 1X PBS) was added to the respective wells (50 μg /well) and incubated for 3–4 h. After incubation with MTT reagent, the MTT reagent was discarded by pipetting without disturbing cells and 100 μL of DMSO was added to rapidly solubilize the formazan. The absorbance was measured at 590 nm.

Table 1
Primers used for the RTPCR reaction.

Gene	Primer pair	Sequence (5' → 3')	Tm	GC %	Expected product size (bp)
HSP90	FP	TGGACAGCAAACATGGAGAG	57.3	50	204
	RP	AGACAGGAGCGCAGTTTCAT	57.3	50	
GAPDH	FP	CGACCACTTTGTCAAGCTCA	58.4	50	238
	RP	CCCCTCTTCAAGGGGTCTAC	58.8	60	

2.2. Gene selection and primer design

The GAPDH was used as housekeeping gene for HEK-293 and MCF-7 cell lines. HEK-293 was used as control cell line for the comparison of gene expression. Primers were designed for GAPDH gene (NG_007073) and HSP90 gene (NM_001017963.2) using Primer– 3 and synthesized for GAPDH and HSP90 at Eurofins Genomics, India. The details of the primers were as tabulated in Table 1.

2.3. RNA isolation

Both MCF-7 and HEK-293 cultured in DMEM medium and subjected to different treatments were washed twice with PBS and to the adherent cells 2 mL of Trizol (per T25 flask) was added and transferred to a falcon tube and vortexed. Samples were allowed to stand for 5 min at room temperature. To this 0.2 mL of chloroform per 1 mL of Trizol was used. The tube was shaken vigorously for 15 s. The tube was allowed to stand at room temperature for 5 min. The resulting mixture was centrifuged at 10,000*g* for 15 min at 4 °C. The colourless upper aqueous phase was transferred to a new clean tube. To this 0.5 mL of isopropanol was added per 1 mL of Trizol used. The mixture was mixed gently and incubated at room temperature for 5 min and centrifuged at 10,000 rpm for 10 min at 4 °C. The supernatant was discarded and the RNA pellet was washed by adding 1 m L of 70% ethanol and mixed properly. The suspension was centrifuged for 5 min at 14,000 rpm at 4 °C and the supernatant was discarded. The pellet was dried by incubating in a dry bath for 5 min at 55 °C. The pellet was then resuspended in 25 μL of DEPC treated water (Table 2).

2.4. cDNA synthesis and semiquantitative RT-PCR

A semi quantitative reverse transcriptase polymerase chain reaction (RT-PCR) was carried out using Techne Prime system to determine the levels of HSP90 and GAPDHmRNA expressions. The cDNA was synthesized from 2 μg of RNA using the Verso cDNA synthesis kit (Thermo Fischer Scientific) with oligo dT primer according to the manufacturer's instructions. The reaction volume was set to 20 μL and cDNA synthesis was performed at 42 °C for 60 min, followed by RT inactivation at 85 °C for 5 min. The PCR mixture (final volume of 20 μL) contained 1 μL of cDNA, 10 μL of Red Taq Master Mix 2x (Amplicon) and 1 μM of each complementary primer specific for HSP90 and GAPDH (internal control) sequence (Table 1). The samples were denatured at 94 °C for 5 min, and amplified using 30 cycles of 94 °C for 30 s, 52 °C for 30 s, and

Table 2
IC_{50} values of *C. flexuosus* oil with different cancer cells.
Adapted from Sharma et al. [10].

Tissue	Cell lines	IC_{50} (μg/mL)
Colon	HT-29	42.4
	HCT-15	60.2
	SW-620	28.1
	502713	4.2
Lung	H-226	61.4
	A-549	49.7
	Hop-62	79
Liver	Hep-2	4.8
	SiHa	6.5
	DU-145	41.4
	KB	50.8
	IMR-32	4.7

72 °C for 1 min for HSP90 and for GAPDH the renaturation was set to 52 °C for 30 s followed by a final elongation at 72 °C for 10 min. The optimal numbers of cycles have been selected for amplification of these two genes experimentally so that amplifications were in the exponential range and have not reached a plateau. 10 μL of the final amplification product were run on a 2% ethidium– stained agarose gel. Quantification of the gene expression was measured by the optical density of the bands, using the imaging program Image J.

2.5. RT-qPCR and gene expression study

A quantitative reverse transcriptase polymerase chain reaction (RT-PCR) was carried out using SYBR green in Stratagene Mx3005P real time PCR to determine the levels of HSP90 and GAPDH mRNA expressions. The cDNA was synthesized from 2 μg of RNA using the Verso cDNA synthesis kit (Thermo Fischer Scientific) with oligo dT primer according to the manufacturer's instructions. The reaction volume was set to 20 μL and cDNA synthesis was performed at 42 °C for 60 min, followed by RT inactivation at 85 °C for 5 min. RT- PCR was done in 20 μL volume containing cDNA (1 μL), forward and reverse primer (1 μL), Sybr green MM (10 μL), DEPC treated water (7 μL). 2step RT PCR reaction temperature profile was 95 °C for 5 min followed by 40 cycles of 95 °C for 30 s, 52 °C for 0.3 min, 72 °C for 1 min and a final extension step at 72 °C for 10 min. Relative expression of target HSP90 gene in relation to housekeeping gene (GAPDH) and untreated control cells were determined by the comparative Ct method (ΔΔ Ct method) described by Schmittgen et al. [20]. The average of Ct values of triplicate data from different groups for HSP90 (target) and GAPDH (reference) genes were used for ΔΔCt [20]. The fold change was calculated by using the formula fold change = $2^{-\Delta\Delta Ct}$.

2.6. Western blotting

The cell lysates were prepared using 20 mM Tris-HCl buffer (pH 8.0) containing 150 mM NaCl, 1% SDS and 1 mM EDTA. The protease inhibitor solution was added at 2 μg/mL concentration. Lysates were sonicated for 10 s, centrifuged for 15 min at 10,000*g* and stored at −80 °C. The protein concentration was measured before loading on the gel, using the BCA protein assay kit from Thermo Fisher (Compat-Able™). 40 μg of protein lysate was mixed with 4X loading buffer. The proteins were separated on 10% SDS-PAGE gel. The gel was electrophoretically transferred to a nitrocellulose membrane. Nitrocellulose blot was blocked with 5% non-fat dry milk in TBST buffer (20 mM Tris-HCl (pH 7.4), 150 mM NaCl, and 0.1% Tween-20) for 1 h and incubated for 4 h with primary antibodies diluted in TBST/5% non-fat dry milk as per the supplier's recommendations (1/1000). Membranes were then washed with TBST for 20 min, 3 times and incubated for 1 h with HRP (horseradish peroxidase) labelled secondary antibodies in TBST. After an additional four washes with TBST, bound antibody was visualized by chemiluminescence.

2.7. Inhibition of HSP90 - ATPase activity

The terpenoids, alkaloids and glycosides are the biomolecules mainly obtained from plant sources naturally. These constitute the major components of folk medicine. The standard compounds like Withaferin a, asiatic acid, lemongrass oil were used at 80 μg/mL for screening the HSP90-ATPase activity inhibition. The HSP90 – ATPase activity inhibition was tested according to the protocol described by Martin et al. using malachite green reagent for colorimetric detection of phosphomolybdate complex [21]. The malachite green reagent was prepared freshly and allowed to stand at room temperature for 2 h before use.

The compounds viz. LO, citral and geraniol were diluted in 100% DMSO and further dilute to get 0.1%DMSO and also to get 400 μg/mL concentrations of the components. 5 μL of the diluted sample was used in the assay (final concentration of 100 μg/mL). The HSP90 (Stressmarq, SPR-102A) was diluted to 0.2 mg/mL using 100 mM Tris buffer (100 mM Tris – HCl, 20 mM KCl, 6 mM $MgCl_2$, pH 7.4). This protein solution (10 μL) was used as HSP90 – ATPase protein in the assay. The colorimetric assay was carried out using 5 μL of diluted components were added in the test reactions. The control reaction was carried out using 5 μL of 0.1% DMSO. 6 mM ATP prepared in Tris - HCl buffer was used as substrate in the revsactions. The reactions were carried out in 96 - well plates in triplicates. The standard graph was prepared from 0 to 8.3 μM of inorganic phosphate using KH_2PO_4. After incubating plate for 3 h at 37 °C (with intermittent shaking) the reaction was stopped using 80 μL malachite green reagent and 10 μL of sodium citrate. The plate was read using microplate reader (Biorad) at 620 nm.

3. Results and discussion

3.1. Cytotoxicity study

The GC–MS analysis was carried out before using it in the cytotoxicity study and the GC–MS graph was as shown in Fig. 1. The cytotoxicity of the LO, citral and geraniol was tested with MCF-7 cells grown in DMEM medium for 24 h without FBS. The compounds were tested from10 μg/mL to 320 μg/mL. When tested using MTT (3 – (4, 5 – dimethythiazol – 2 – yl) – 2, 5– diphenyl tetrazolium bromide) assays, all 3 compounds inhibited the proliferation of MCF-7 cell lines with LO showing the highest inhibition (IC_{50} = 69.33 μg/mL). Citral (IC_{50} = 140.7 μg/mL) and geraniol (IC_{50} = 117 μg/mL) also inhibited the cell proliferation with less efficiency as shown in Fig. 2b whereas with HEK-293 cells (Fig. 2a), the values were LO (80.19) citral (IC_{50} = 174.5 μg/mL) and geraniol (IC_{50} = 138.9 μg/mL). Apparently MCF-7 cells were more susceptible to the lemongrass oil, citral and geraniol treatments. The results were as shown in Fig. 2(a & b).

The results of cytotoxic studies showed that lemongrass oil had the highest inhibition of both HEK-293 and MCF-7. The IC_{50} values were 80.19, 138.9 and 174.5 μg/mL for lemongrass oil, geraniol and citral with HEK-293 cells. It was 69.33, 117 and 140.7 μg/mL for lemongrass oil, geraniol and citral with MCF-7 cells. The lemongrass oil was the most effective among the 3 components considered for the study with respect to toxicity to both the cells, 80.19 μg/mL with HEK-293 and 69.33 μg/mL with MCF-7. The citral and geraniol inhibited the proliferation at comparatively high concentrations compared to lemongrass oil as shown in Fig. 2(a & b).

3.2. cDNA synthesis and semiquantitative RT-PCR

The HEK-293 and MCF-7 cells were treated with the selected low and high concentrations i.e. 80 and 160 μg/mL of the lemongrass oil, citral and geraniol. The gene selection and primer design was done as

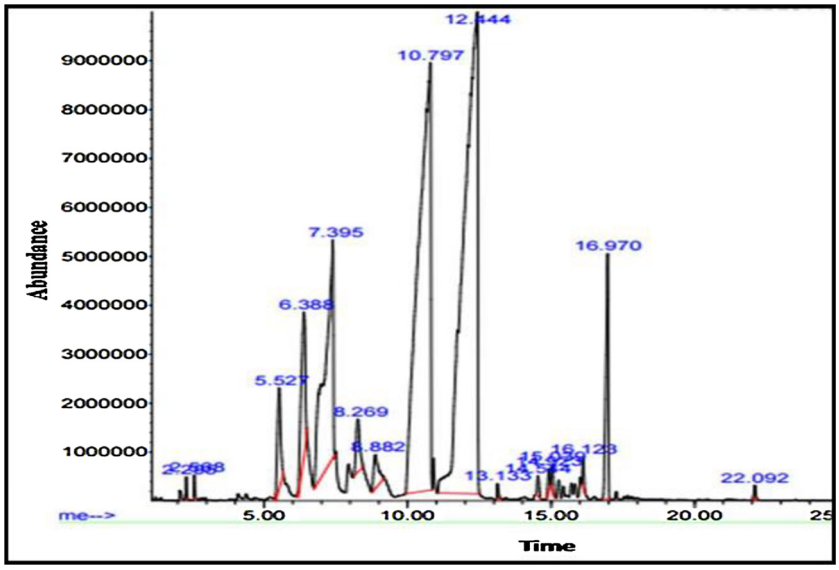

Fig. 1. GC–MS analysis of *C. flexuosus* essential oil.

explained in the Section 2.2. The semiquantitative PCR was carried out as described in the Section 2.4. The gel doc images of cDNA were as shown in Fig. 3(a–c). The intensity of the bands was analysed using Image J software. The results of the analysis were as shown in Fig. 3(a–c). The fold change of gene expression from the semi-quantitative RT-PCR results were calculated normalizing the value of HSP90 gene expression to the GAPDH expression. The results indicate the lemongrass oil is inhibiting the HSP90 gene expression in HEK-293 and MCF-7 with comparatively higher inhibition in MCF-7 cells. HSP90 gene expression was significantly affected at 80 µg/mL (0.55 fold) and at 160 µg/mL was five times lesser (0.22 fold) compared to control as shown in Fig. 3a. Further to confirm these observations a quantitative RT-PCR was carried out.

3.3. HSP90 gene expression quantification by RT-qPCR

The quantitative RT-PCR reaction was carried out as described in Section 2.5 and the fold change of gene expression was calculated using $2^{-\Delta\Delta Ct}$ method. The experiments were done three times and the results were as shown in Fig. 4. The error bars represent the standard deviation of the triplicate data. The fold change of HSP90 gene expression was calculated by keeping GAPDH as internal control. The results showed that lemongrass oil at 80 µg/mL and 160 µg/mL reduced the expression of the HSP90 gene significantly in MCF-7 compared to HEK-293 cells as shown in Fig. 4.

3.4. Western blotting

The expression of HSP90 protein in the different treatments were analysed using western blotting as described in Section 2.6. The β-actin was used as internal control. The bands observed as shown in Fig. 5a.

The intensity of the bands was assessed by Image J software. The results were as shown in Fig. 5(b & c). There was comparatively reduced in different treatments of lemongrass oil. The experiments were carried out using fresh samples of cells which were treated with different compounds and dosages. The cell extract kept in storage for 1 week time showed complete absence of HSP90 bands in the treatment samples. So fresh samples were used for the study. The intensity analysis of the bands showed HSP90 protein was found to be present at 75% in 80 µg/mL treatment and 53% in 160 µg/mL in MCF-7 compared to control. In HEK-293 cells it was 89% in 80 µg/mL and 77% present in 160 µg/mL compared to control. This confirms LO is inhibiting the HSP90 expression at protein level also with a higher inhibition observed in cancer cells compared to normal cells (Fig. 6).

3.5. Inhibition of HSP90–ATPase activity

The gene expression and protein expression studies showed that the

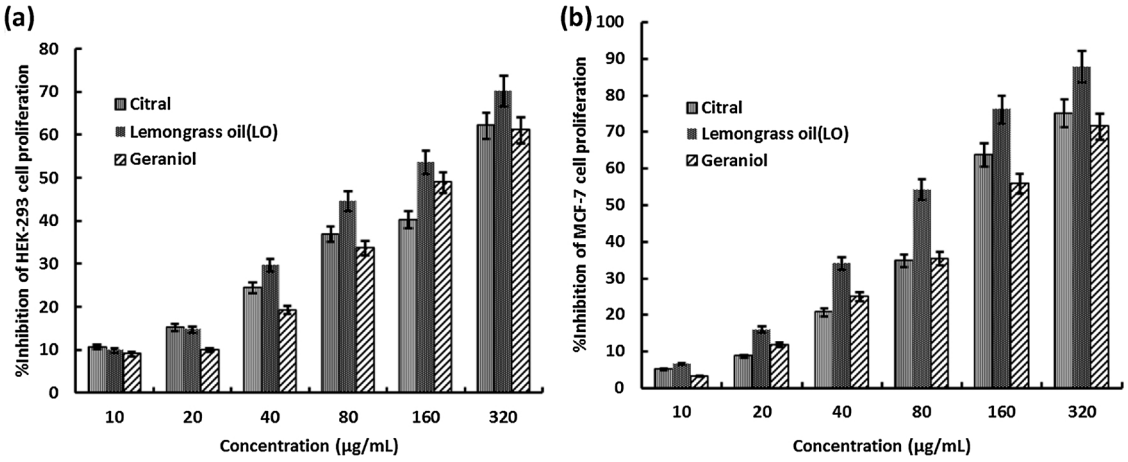

Fig. 2. Cytotoxicity of citral, lemongrass oil (LO) and geraniol with a.HEK-293 cells, and b. MCF-7 cells.

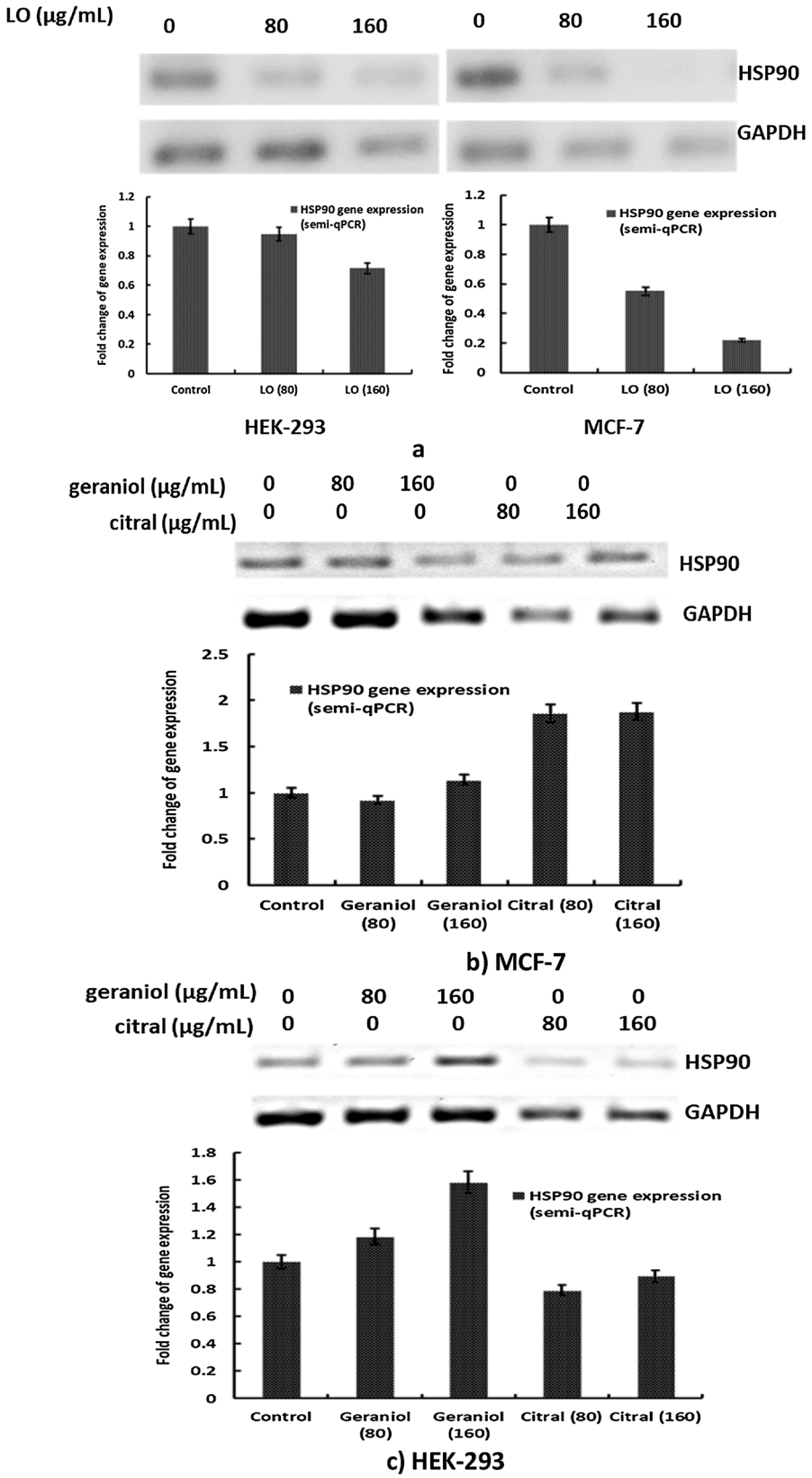

Fig. 3. Effects of LO, citral and geraniol on the expression of HSP90 GAPDH in HEK-293 and MCF-7 cells. a) LO (80 and 160 µg/mL) on HEK-293 and MCF-7, b) citral (80 and 160 µg/mL) and geraniol (80 and 160 µg/mL) on MCF-7, c) citral (80 and 160 µg/mL) and geraniol (80 and 160 µg/mL) on HEK-293.

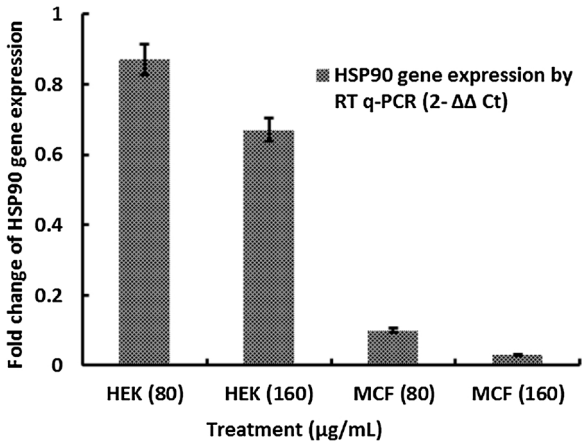

Fig. 4. HSP90 mRNA expression levels relative to GAPDH mRNA levels under treatment stress of selected lemongrass oil concentrations analyzed by real-time quantitative RT-PCR.

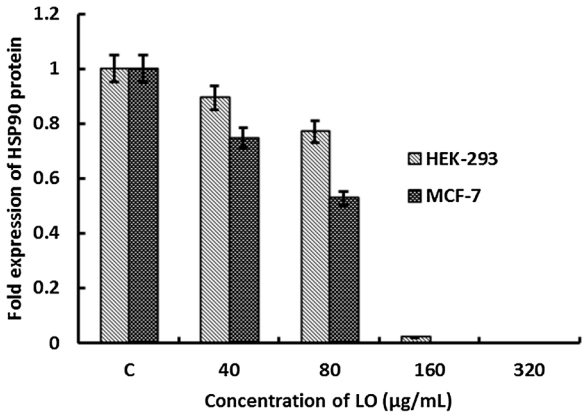

Fig. 6. HSP90 gene expression by RT-qPCR at different treatment concentrations of LO.

essential oil inhibits the HSP90 in HEK-293 and MCF-7 cells. To study the in vitro functional roles of the essential oil and its components citral and geraniol in inhibiting the HSP90-ATPase activity we performed HSP90-ATPase activity inhibition assessment by following the high throughput colorimetric screening protocol reported by Rowlands et al. [21] as discussed in the Section 2.7. The HSP90-ATPase activity using pure human HSP90 (SPR102A, Stress Marque Biosciences, Canada). The order of HSP90 - ATPase activity inhibition was: Withaferin A > lemongrass oil > citral > geraniol > asiatic acid. The results were as shown in Fig. 7a.

Further the LO was tested at 4 concentrations 20 µg/mL, 40 µg/mL, 80 µg/mL, 160 µg/mL in duplicates. The results were as shown in Fig. 7b. The inhibition of the HSP90-ATPase activity was observed from 40 µg/mL of LO. The inhibition was enhanced in 80 µg/mL and 160 µg/mL.

4. Discussion

The present study reports the cytotoxicity, the inhibition of HSP90 at both gene and protein level by lemongrass oil significantly in the cancer cells. The cytotoxicity studies showed the lemongrass oil was highly cytotoxic (IC_{50} = 69.33 µg/mL). The IC_{50} values were reported with the essential oil of *C. flexuosus* with different cancer cell lines by Sharma et al. [10]. The same essential oil was found to be inhibitory to the HSP90-ATPase activity also in our study.

The gene expression studies showed that the essential oil of *C.*

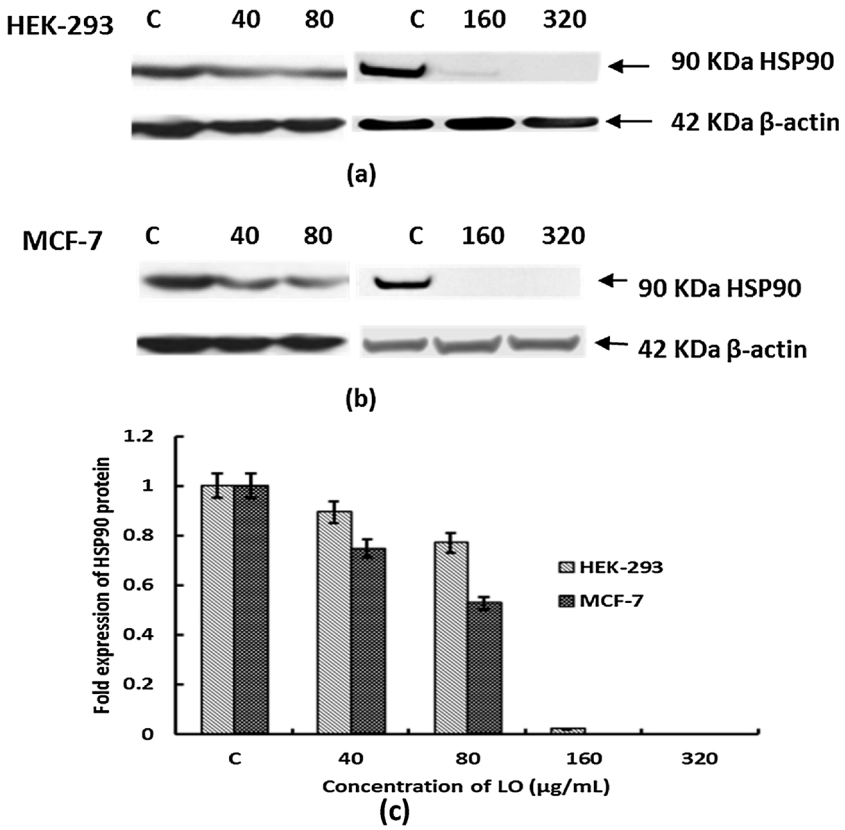

Fig. 5. Western blot analysis fold expression of HSP90 calculated using the β-actin as an internal standard. a) HEK-293 cells treated with C – Control (0 µg/mL), 40,80,160 and 320 µg/mL lemongrass oil b) MCF-7 cells treated with C – Control (0 µg/mL), 40, 80, 160 and 320 µg/mL lemongrass oil.

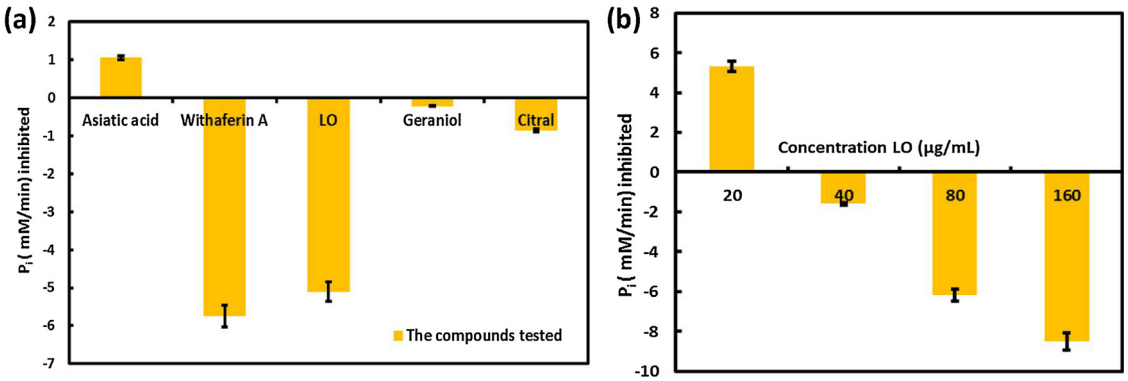

Fig. 7. HSP90-ATPase activity inhibitory activity a) screening of plant based compounds, b) inhibition by LO at different concentration.

flexuosus (LO) showed inhibition of HSP90 in both HEK-293 and MCF-7 cells. Citral showed 20% inhibition in 80 µg/mL and 10% inhibition in 160 µg/mL in MCF-7 cells. However it showed increase in the mRNA expression of HSP90 in HEK-293 cells. Geraniol showed no inhibition in either HEK-293 or MCF-7; however the HSP90 expression was significantly increased in MCF-7 cells. Considering these outcomes, further studies of quantification of gene expression by RT-qPCR and quantification of protein expression was carried out using LO. The RT-qPCR studies confirmed the indications of semiquantitatice RT-PCR that LO inhibited the HSP90 gene expression to a great extent (90% with 80 µg/mL and 97% in 160 µg/mL) with LO treatment as calculated by ΔΔ Ct method.

Withania somnifera contains Withaferin A which is reported to inhibit the Hsp90/Cdc37 chaperone/co-chaperone complex [20]. Also Withaferin A was reported to be very promising anticancer compound in pancreatic cancer and B-cell lymphomas by HSP90-ATPase activity [22,23]. Another pentacyclic triterpene asiatic acid is reported to protect human bronchial 16HBE and BEAS-2B cells against hydrogen peroxide induced oxidative and inflammatory injury [24]. Asiatic acid is reported to show antioxidant, anti-inflammatory and protection against glutamate or β-amyloid induced toxicity [25]. In our study we included Withaferin A and asiatic acid for studying the HSP90 inhibition alongwith the essential oil from *C. flexuosus* and its components citral and geraniol. The results showed the HSP90-ATPase activity was inhibited significantly with both LO and Withaferin A as shown in Fig. 7a. But asiatic acid showed increased HSP90-ATPase activity. The inhibition observed with citral and geraniol were not significant compared to LO or Withaferin A. The study with different concentrations of LO showed the ATPase activity was inhibited by LO from 40 µg/mL to 160 µg/mL (Fig. 7b). However, it is unclear which component of the LO is responsible for the HSP90-ATPase activity inhibition. Because the major components of the lemongrass oil viz. citral and geraniol did not show HSP90-ATPase activity in vitro. Also, it could be possible that the synergy of the different components is responsible for the HSP90-ATPase activity inhibition. To separate the components of LO and evaluate the mechanism of binding of those components into the N-terminal or C-terminal domains of HSP90 to study the interactions would be the future perspective for this study.

5. Conclusion

The essential oil of *C. flexuosus* showed higher cytotoxicity to the MCF-7 cells and HEK-293 cells with the higher cytotoxicity for MCF-7 cells. The components citral and geraniol failed to inhibit the HSP90 gene expression. However the essential oil LO was also shown to inhibit the gene and protein expression. Also the LO showed inhibition of HSP90-ATPase activity.

Transparency document

The Transparency document associated with this article can be found in the online version.

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