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Exploring the Anti-Cancer Effects of Kerra Extract on EGFR-Driven Lung Cancer Through Kinase Inhibition and FTIR Analysis

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Corresponding Author:	kiattawee choowongkomon, Ph.D. Kasetsart University Faculty of Science Bangkok, THAILAND
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Kasetsart University Faculty of Science
Corresponding Author's Secondary Institution:	
First Author:	Thomanai Lamtha, Ph.D.
First Author Secondary Information:	
Order of Authors:	Thomanai Lamtha, Ph.D.
	Buabarn Kuaprasert, Ph.D.
	Nutnaree Kumsiri, M.Sc.
	Sitthinan Somkhuan, B.Sc.
	Wichunee Thongpueg, B.Sc.
	Siriwan Saehlee, M.Sc.
	Kiattawee Choowongkomon, Ph.D.
Order of Authors Secondary Information:	
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Suggested Reviewers:	Jeanette M. Andrade, Ph.D., R.D.N., L.D.N., F.A.N.D. jandrade1@ufl.edu

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	Guillermo Escalante, PhD gescalan@csusb.edu

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Exploring the Anti-Cancer Effects of Kerra Extract on EGFR-Driven Lung Cancer Through Kinase Inhibition and FTIR Analysis

Thomanai Lamtha[1], Buabarn Kuaprasert[2], Nutnaree Kumsiri[3], Sitthinan Somkhuan[3], Wichunee Thongpueg[3], Siriwan Saehlee[3], and Kiattawee Choowongkomon[3,*]

Affiliations:

[1]Department of Clinical Chemistry, Faculty of Medical Technology, Nation University, Lampang 52000, Thailand; thomanai_lam@nation.ac.th (T.L.)

[2]Research and Facility Division, Synchrotron Light Research Institute (Public Organization), Nakhorn Ratchasima 30000, Thailand; buabarn@slri.or.th (B.K.)

[3]Department of Biochemistry, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand; nutnaree.kum@ku.th (N.K.); sittinan4026@gmail.com (S.S.K.); wichuda.th@ku.th (W.T.); siriwan.sah08@gmail.com (S.S.); kiattawee.c@ku.th (K.C.)

[*] **Corresponding author:** Kiattawee Choowongkomon, Department of Biochemistry, Faculty of Science, Kasetsart University, Bangkok, 10900, Thailand.

E-mail address: kiattawee.c@ku.th; **Phone No:** +662-562-5444 Ext 2046

Abstract: Lung cancer, particularly non-small cell lung cancer (NSCLC), remains one of the most prevalent and deadly cancers, with the epidermal growth factor receptor (EGFR) playing a central role in its progression. Despite the clinical success of EGFR-targeted therapies, acquired resistance, notably through mutations like T790M and C797S, presents significant therapeutic challenges. In response to these issues, there is a growing interest in natural compounds that could offer novel mechanisms of action. This study investigates the anti-cancer potential of Kerra™ extract, a traditional Thai herbal remedy, against EGFR-driven lung cancer, focusing on its ability to inhibit EGFR tyrosine kinase activity and its effects on cancer cell migration and metastasis. Through kinase inhibition assays, Kerra™ extract was found to inhibit wild-type and drug-resistant mutant EGFR variants, including L858R/T790M and L858R/T790M/C797S. *In vitro*, Kerra™ extract demonstrated moderate anti-proliferative effects on A549 lung cancer cells (IC_{50} = 386.5 µg/mL) and significantly inhibited cell migration in a wound healing assay. Additionally, Fourier Transform Infrared (FTIR) spectroscopy revealed distinct biochemical changes in treated cells, including alterations in lipid and protein secondary structures, suggesting that Kerra™ extract induces unique molecular responses compared to conventional therapies like Osimertinib. These findings support the potential of Kerra™ extract as a novel natural compound in overcoming EGFR-TKI resistance and inhibiting lung cancer metastasis.

Keywords: Kerra™ extract, EGFR, lung cancer, tyrosine kinase inhibition, FTIR analysis

1. Introduction

Lung cancer remains one of the leading causes of cancer-related mortality globally, with non-small cell lung cancer (NSCLC) accounting for approximately 85% of all cases(1,2). Among the various molecular targets identified in NSCLC, the epidermal growth factor receptor (EGFR) plays a pivotal role in the regulation of cell growth, differentiation, and survival(3). EGFR, a member of the ErbB family of receptor tyrosine kinases (RTKs), is frequently overexpressed or mutated in many cancers, including lung

cancer, and its dysregulation is often associated with tumorigenesis and poor prognosis(4,5). Activating mutations in EGFR, such as L858R in exon 21 and the T790M mutation in exon 20, are well known to enhance receptor signaling, thereby promoting oncogenic transformation(6). Furthermore, the emergence of resistance mutations, including C797S, has complicated the long-term efficacy of EGFR-targeted therapies, such as first- and second-generation tyrosine kinase inhibitors (TKIs), including Erlotinib, Gefitinib, and Osimertinib(7,8).

First- and second-generation TKIs, which inhibit EGFR tyrosine kinase activity, have provided significant clinical benefits for patients with EGFR-driven NSCLC(9). However, despite initial tumor responses, acquired resistance due to secondary mutations, such as T790M, is inevitable, limiting the therapeutic effectiveness of these agents(10,11). Osimertinib, a third-generation EGFR TKI, has been developed to target both the activating EGFR mutations and the T790M resistance mutation(12). However, resistance to Osimertinib due to the C797S mutation has been reported in clinical settings, underscoring the urgent need for novel therapeutic strategies to overcome these resistance mechanisms(13). Given these challenges, there is increasing interest in exploring alternative treatment strategies, such as the use of natural compounds and traditional herbal remedies, to complement or bypass the limitations of conventional EGFR-targeted therapies.

Traditional herbal medicines have long been utilized in various cultures for their therapeutic potential. In particular, several plant-derived compounds have been shown to possess anti-cancer properties by targeting key signaling pathways involved in tumorigenesis, such as EGFR(14-16). Kerra™ extract, derived from a traditional Thai herbal formulation, has shown promise as a potential anti-cancer agent due to its reported anti-inflammatory, anti-oxidant, and anti-tumor effects(17-20). While the specific bioactive compounds in Kerra™ extract remain to be fully elucidated, preliminary studies have demonstrated its ability to inhibit various cancer-related pathways, including those involving EGFR and its downstream signaling networks. The extract has been shown to exert multi-faceted effects, including the inhibition of EGFR tyrosine kinase activity, suppression of cell migration, and modulation of apoptosis, which are crucial factors in the progression and metastasis of lung cancer(18,19).

Recent studies have suggested that Kerra™ extract may be particularly effective in inhibiting the activity of drug-resistant EGFR mutants, which are commonly implicated in the failure of current EGFR-targeted therapies. *In vitro* studies have reported that Kerra™ extract effectively reduces the viability of cancer cells harboring EGFR mutations, including the T790M mutation, which is known to confer resistance to first- and second-generation EGFR inhibitors(11,21). Furthermore, the extract has been shown to modulate key cellular processes, such as cell migration and invasion, which are essential for cancer metastasis and poor prognosis(22).

Given the rising incidence of EGFR-driven lung cancers and the limitations of current EGFR-targeted therapies, the exploration of alternative, natural compounds, such as Kerra™ extract, offers significant potential for improving treatment outcomes. The aim of this study is to assess the inhibitory effects of Kerra™ extract on EGFR tyrosine kinase activity, particularly in the context of drug-resistant mutant variants (L858R/T790M and L858R/T790M/C797S), and to evaluate its impact on lung cancer cell migration and metastasis. Furthermore, we will utilize Fourier Transform Infrared Spectroscopy (FTIR) to profile the biochemical changes induced by Kerra™ extract treatment, providing deeper insights into its molecular mechanisms. This study seeks to contribute to the growing body of evidence supporting the

therapeutic potential of Kerra™ extract as a novel, natural-based strategy for targeting EGFR-driven lung cancer and overcoming resistance to existing therapies.

2. Materials and Methods

2.1. Kinase Inhibition Assay

The inhibitory activity of Traditional Thai Herb (Kerra™) Extract against the tyrosine kinase domain of wild-type EGFR (wt-EGFR), double mutant EGFR (L858R/T790M), and triple mutant EGFR (L858R/T790M/C797S) was assessed using the ADP-Glo™ Kinase Assay (Promega, USA). The assay was performed following the manufacturer's protocol and previous studies(23,24). Reactions were conducted in a 384-well Greiner Bio-One Lumitrac solid white plate and incubated at room temperature for 1 hour. Luminescence was measured using a Synergy HTX Multi-Mode microplate reader (BioTek, USA). The half-maximal inhibitory concentration (IC_{50}) values were determined using nonlinear regression analysis of the log-transformed compound concentrations versus percentage control activity in GraphPad Prism 8 (GraphPad Software Inc., USA). Each experiment was performed in triplicate to ensure reproducibility.

2.2. Cell Culture

The EGFR-positive human lung cancer cell line (A549) was obtained from Biomedia (Thailand) Co., LTD and cultured in DMEM (Gibco, USA) supplemented with 10% FBS (ATCC, USA) and 100 U/mL penicillin-streptomycin (Sigma-Aldrich, USA). Cells were maintained in a humidified incubator at 37°C with 5% CO_2, with the medium changed every 48 hours. At 80–90% confluence, cells were passaged using 0.25% trypsin-EDTA (Gibco, USA) under sterile conditions in a biosafety cabinet. Cell morphology and confluency were routinely monitored using an inverted phase-contrast microscope.

2.3. MTT Cell Viability Assay

MTT cell viability assay was performed as described in our previous publications(16,25). Briefly, A549 cells (5×10^3 cells/well) were seeded in 96-well plates and incubated for 24 h. The cells were then treated with different concentrations of the Kerra™ extract for 72 h. Next, the MTT reagent and medium were added to each well at a ratio of 1:10, followed by incubation at 37°C for 3 h. The medium was then removed and 50 µL of DMSO was added to each well for solubilization of formazan. The absorbance was measured at 570 nm using a microplate reader (Synergy HTX Multi-Mode reader, BioTek, USA). IC_{50} values were determined by comparing the absorbance of treated wells to the untreated control using the GraphPad Prism8 software (GraphPad Software Inc., USA). The experiment was done in triplicate.

2.4. Scratch Wound Assay and Image Analysis

The scratch wound assay was performed to assess the effect of Traditional Thai Herb (Kerra™) Extract on the migration of A549 lung cancer cells. A549 cells were seeded in 24-well plates (3.5×10^5

cells/well) and cultured until they reached 90% confluence. A sterile 200-µL pipette tip was used to create a uniform scratch in the cell monolayer, after which the wells were washed with phosphate-buffered saline (PBS) to remove detached cells. The cells were then treated with Kerra™ extract at concentrations of 150, 300, 600, and 1,000 µg/mL, while an untreated control (serum-free medium only) and 10 µM Osimertinib (positive control) were included for comparison. The wound area was monitored, and images were captured at 0-, 24-, and 48-hours post-treatment using an inverted phase-contrast microscope. The percentage of wound closure was analyzed using ImageJ software. The wound area was measured in each image, and the percentage of closure was calculated using the formula:

$$\% \text{ Wound Closure} = \frac{\text{Initial wound area} - \text{Remaining wound area}}{\text{Initial wound area}} \times 100$$

All experiments were conducted in triplicate, and data were statistically analyzed to evaluate the impact of Kerra™ extract on A549 cell migration.

2.5. Fourier Transform Infrared Spectrometer (FTIR)

For FTIR analysis, A549 lung cancer cells were seeded at a density of 36,000 cells per well in 6-well plates and incubated at 37°C with 5% CO_2 for 24 hours. The medium was then replaced with one of the following treatments: DMEM (untreated control), 1% DMSO, Osimertinib at its IC_{50} concentration (4.82 µg/mL), or Kerra™ extract at its IC_{50} concentration (386.5 µg/mL). Cells were further incubated under the same conditions for 72 hours. After treatment, cells were harvested using 0.25% Trypsin-EDTA and washed twice with PBS. The cells were then fixed with 4% formalin for 30 minutes, with gentle tapping every 5 minutes, followed by two washes with 0.9% saline to remove debris and residual salts. The cell pellets were washed three times with distilled water, and a final 10 µL suspension was mixed thoroughly, dropped onto a glass slide placed over a barium fluoride (BaF_2) infrared window, and air-dried for FTIR analysis. FTIR sample measurements were performed using the transmission mode of FTIR microspectroscopy with a Bruker Vertex 27 spectrometer, coupled with a Bruker Hyperion 3000 microscope and a mercury cadmium telluride (MCT) detector cooled by liquid nitrogen (Bruker Optics; Ettlingen, Germany). FTIR spectral data collection was controlled using OPUS software version 8.5 (Bruker Optics Ltd.; Ettlingen, Germany), with the following parameters: scanning range of 4000 to 600 cm^{-1}, IR beam aperture of 50×50 square microns, resolution of 6 cm^{-1}, and scanning time of 64. FTIR spectra, with amide I intensity, were extracted from every single image, and spectra outside the 0.2 to 1.2 absorption range were excluded using the OPUS program. Principal Component Analysis (PCA) of the FTIR biochemical profiles of cells before and after treatment with herbal extract and Osimertinib was performed using Unscrambler X software version 10.5 (CAMO; Oslo, Norway), with 7 smoothing points and EMSC applied for the analysis.

2.6. Statistical Analysis

Wound closure was quantified as a percentage, with the initial wound area at time zero set as 0% closure. Statistical analysis was conducted using one-way ANOVA, followed by Bonferroni post-hoc correction for multiple comparisons, with untreated cells serving as the control. Data are presented as mean

± standard deviation (SD), and differences were considered statistically significant at $p < 0.05$. All graphs and statistical analyses were generated using GraphPad Prism 8.0 (GraphPad Software Inc., USA).

3. Results

3.1. EGFR- tyrosine kinase inhibitory activity of Kerra

The EGFR-tyrosine kinase inhibitory activity of Kerra™ extract was evaluated using the ADP-Glo Kinase Assay Kit (Promega, USA) to assess its potential as an EGFR-targeting agent. The results demonstrated that Kerra™ extract effectively inhibited the tyrosine kinase activity of wt-EGFR with an IC_{50} of 329.8 ± 22.1 µg/mL (**Fig. 1a**), while also showing inhibitory activity against drug-resistant mutant EGFR variants, including double mutant EGFR L858R/T790M (IC_{50} = 540.1 ± 12.7 µg/mL)(**Fig. 1b**) and triple mutant EGFR L858R/T790M/C797S (IC_{50} = 522.6 ± 23.1 µg/mL) (**Fig. 1c**). Although the inhibitory potency of Kerra™ extract was strongest against wild-type EGFR, its ability to suppress the activity of mutant EGFR-TKs is particularly notable, given that these mutations are associated with resistance to conventional tyrosine kinase inhibitors (TKIs). Interestingly, while the IC_{50} values for mutant EGFR-TKs were higher compared to wt-EGFR, the extract still demonstrated measurable activity, suggesting a potential role in overcoming EGFR-TKI resistance. These findings highlight the dual inhibitory potential of Kerra™ extract, targeting both wild-type and resistant mutant EGFR-TKs, which could have significant implications for the treatment of EGFR-driven cancers. Further studies are warranted to elucidate the underlying molecular mechanisms, optimize its efficacy, and evaluate its therapeutic potential in cancer models with EGFR overexpression and resistance-associated mutations.

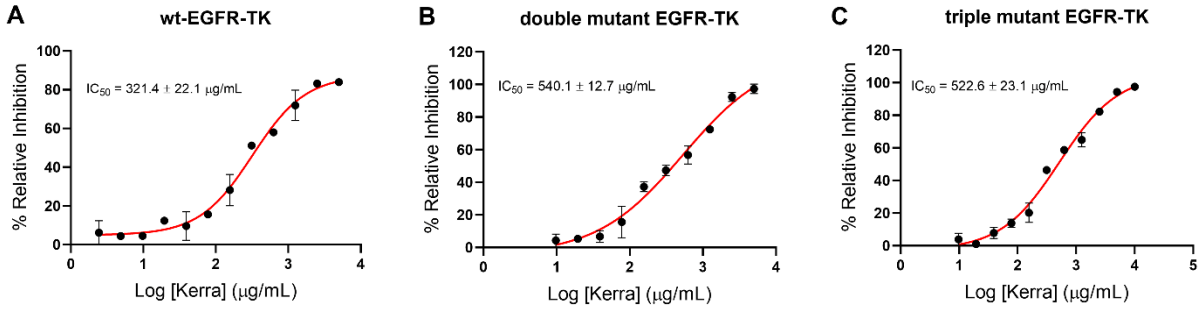


Fig. 1 *In vitro* kinase inhibitory activity of Kerra™ extract against EGFR-TK. The tyrosine kinase inhibition activity of (**A**) wt-EGFR, (**B**) double mutant EGFR L858R/T790M, and (**C**) triple mutant EGFR L858R/T790M/C797S by Kerra™ extract was determined using the ADP-Glo Kinase Assay Kit (Promega, USA). Graph was plotted as percentage inhibition of kinase activity versus log of dose, estimated using GraphPad Prism version 8.0. (GraphPad Software Inc., USA). The half maximal inhibitory concentration (IC_{50}) values are given in in the graph, Dose-response data points represent the mean ± SD (n = 3)

3.2. Anti-EGFR-positive cancer activity of Kerra™ extract

Given the previously demonstrated strong inhibitory effects of Kerra™ extract against both wild-type and mutant forms of EGFR-TK, its potential to suppress the proliferation and metastatic capabilities

of cancer cells overexpressing EGFR was further examined in this study. To assess whether the EGFR-TK inhibitory properties of Kerra™ translate into actual anti-cancer effects in an EGFR-overexpressing model, the MTT cell viability assay was employed to evaluate the growth inhibitory activity of Kerra™ extract in comparison to Osimertinib, a well-established EGFR tyrosine kinase inhibitor, using the A549 lung cancer cell line. The results revealed that Osimertinib exerted potent cytotoxic effects with an IC_{50} value of 9.6 ± 0.4 µM or 4.8 µg/mL (**Fig. 2a**), whereas Kerra™ extract also demonstrated a measurable anti-proliferative effect with an IC_{50} value of 386.5 ± 15.9 µg/mL (**Fig. 2b**). Although the cytotoxic potency of Kerra™ was lower than that of Osimertinib, the dose-dependent reduction in cell viability observed suggests that Kerra™ extract is capable of inducing significant anti-cancer activity at higher concentrations. These findings support the notion that Kerra™ extract possesses promising anti-lung cancer potential, likely mediated through its inhibition of EGFR-TK signaling pathways.

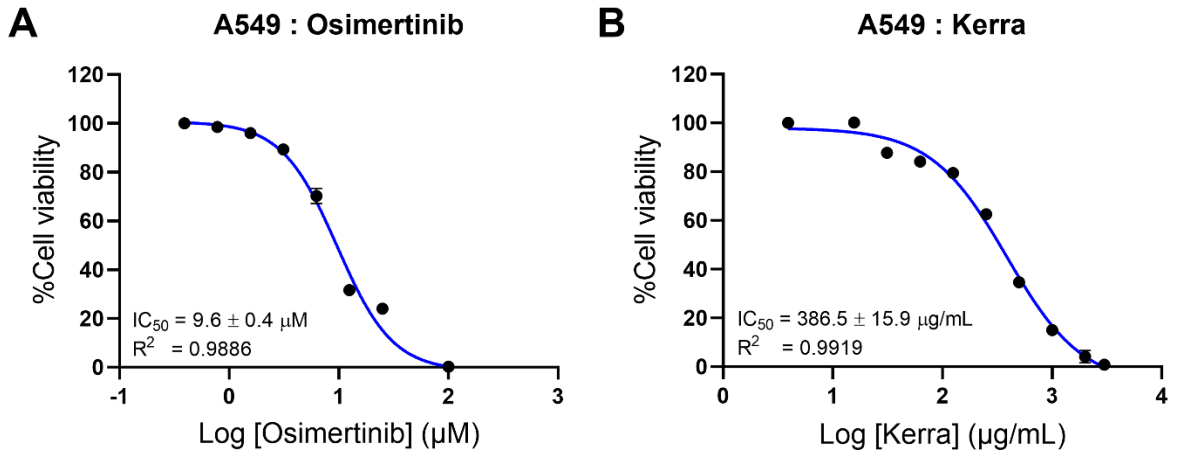


Fig. 2 Effect of Osimertinib and Kerra™ extract on A549 lung cancer cell viability. A549 cells were treated for 72 hours with varying concentrations of (**A**) Osimertinib and (**B**) Kerra™ extract, and cell viability was assessed using the MTT assay. Dose-response curves were generated using GraphPad Prism version 8.0 (GraphPad Software Inc., USA), with concentrations plotted in log10 (µg/mL). Data are presented as mean ± SD from three independent experiments (n = 3)

3.3. Kerra™ extract reduced the migration of A549 cells in vitro

The scratch wound assay is a widely used *in vitro* technique to study cell migration and overall cell motility, often in the context of tissue regeneration, cancer metastasis and drug testing(26). The anti-migratory effects of Kerra™ extract on EGFR-positive A549 lung cancer cells were assessed using a scratch wound healing assay over 24- and 48-hour periods (**Fig. 3a**). The untreated control group exhibited substantial wound closure, with 75.7 ± 0.6 % closure at 24 hours and 93.6 ± 5.7 % at 48 hours, reflecting the intrinsic high migratory capacity of A549 cells. In contrast, the positive control, Osimertinib, an established EGFR-tyrosine kinase inhibitor, significantly inhibited cell migration, resulting in wound

closure rates of only 19.9 % ± 11.9 at 24 hours and 35.8 ± 18.1 % at 48 hours. Treatment with Kerra™ extract demonstrated a clear concentration-dependent suppression of cell migration. At 24 hours, wound closure decreased progressively with increasing concentrations of the extract: 71.4 ± 6.9 % at 150 µg/mL, 63.5 ± 3.3 % at 300 µg/mL, 56.3 ± 11.0 % at 600 µg/mL, and 48.1 ± 4.1% at 1,000 µg/mL. This inhibitory trend was similarly observed at 48 hours, with wound closure rates of 87.5 ± 4.5 %, 73.6 ± 7.4%, 66.7 ± 1.7 %, and 55.0 ± 15.8 % corresponding to the same concentrations. Notably, the highest concentration of Kerra™ extract (1,000 µg/mL) demonstrated a marked reduction in migration relative to the untreated control, approaching the efficacy of Osimertinib at both time points (**Fig. 3b**). These results indicate that Kerra™ extract effectively impairs A549 cell migration in a dose-dependent manner. While the inhibitory effect was less pronounced than that of Osimertinib, the progressive reduction in wound closure with increasing extract concentration suggests a potential mechanistic link to EGFR pathway modulation. These findings support the potential utility of Kerra™ extract as a complementary or alternative therapeutic candidate for targeting EGFR-driven lung cancer cell migration.

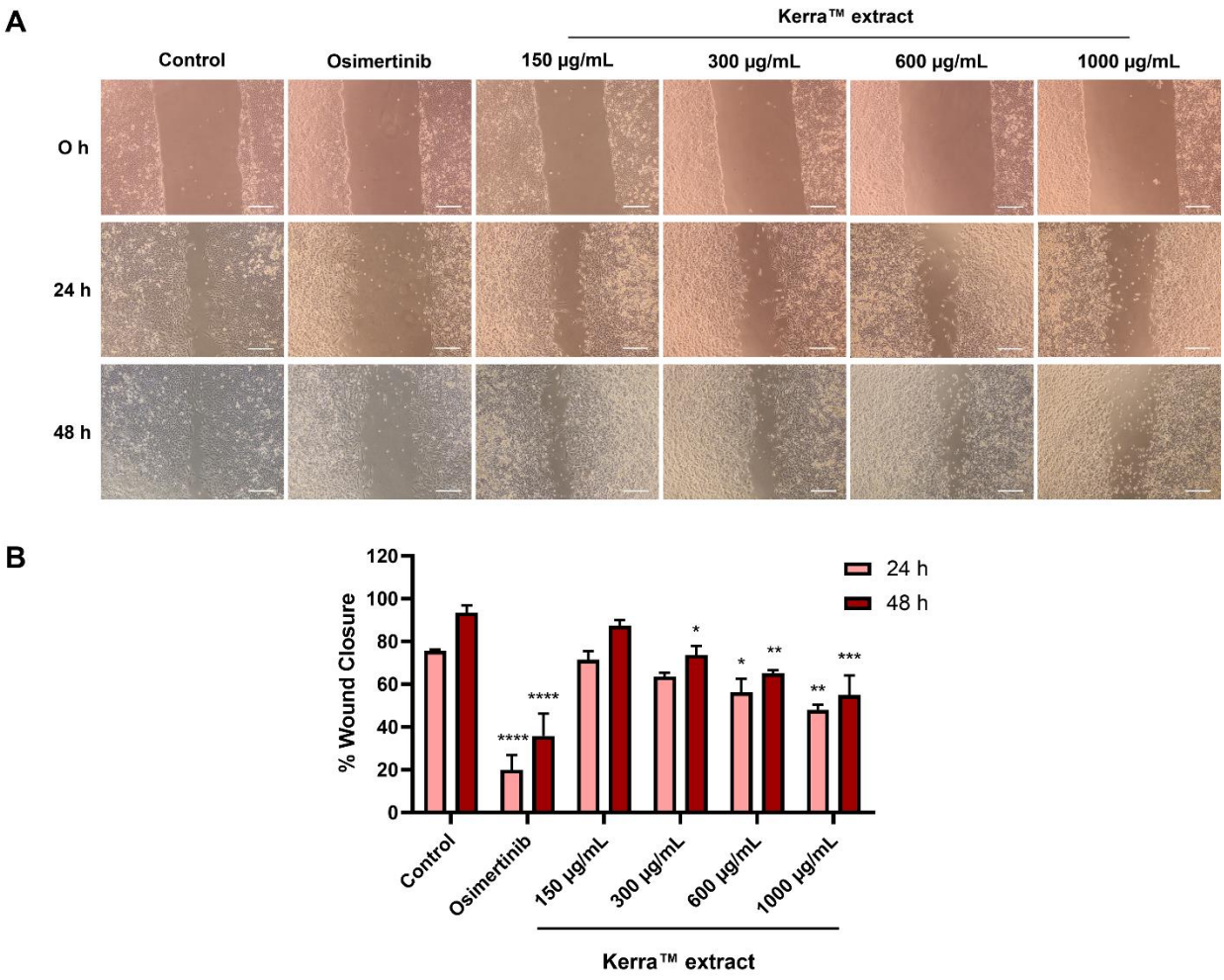


Fig. 3 Wound healing assay of Kerra™ extract exposure. (**A**) Images were captured at 0-, 24-, and 48-hours post-treatment using an inverted phase-contrast microscope (10X magnification). Scale bar is 100 µm in all

images. (**B**) Bar graph showing the percentage of wound closure at 24- and 48-hours following exposure to various concentrations of Kerra™ extract, compared to untreated control and 10 µM Osimertinib. To assess the scratch by quantifying the areas occupied by the lesion, the images were analyzed using the Image J software. Data are presented as mean ± SD (n = 3), and differences were considered statistically significant at $p < 0.05$. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$ versus control). GraphPad Prism version 8.0 (GraphPad Software Inc., USA) was used for statistical analysis

3.4. FTIR profile of Kerra™ extract on A549 lung cancer cells

Transmission FTIR microspectroscopy was used to access biochemical compositions changes of the untreated cell and the cell treated with Osmiertinib and Kerra™ extract. Spectra from the FTIR microspectroscopic analysis with amide I absorption band intensity in the 0.2 to 1.2 absorption range were selected from the untreated and treated cell line individually for further PCA analysis. For overall spectral comparison the FTIR spectra of each treatement group were aligned over the spectral range 3000 to 1000 cm^{-1}. They were taken 7 smoothing points, averaged, normallized and overlaid (**Fig. 4a**). Qualified FTIR spectral groups measured from the untreated cell (68 points) and the cell treated with Osmiertinib (71 points) and Kerra™ extract (77 points) were used for identifying their biomolecular alterations by PCA analysis. The score plot of PC-2 (13%) against PC-4 (3%) (**Fig. 4b**) shows clusters of the three samples which are discriminated by their variables related to the loading plot, especially for the asymmetric and symmetric stretching of $-CH_2-$ fatty lipid at 2917 and 2850, amide I at 1646 and 1612 cm^{-1} (**Table 1** and **Fig. 4c**). To observe biomolecular alteration in the cell after treated with Osmiertinib and Kerra™ extract, averaged secondary FTIR spectra of the three samples, taken from the EMSC step, were ploted and overlaid, and the loadings results were used as guide directions for the interpretation. In the lipid region, two peaks at 2960 and 2852 cm^{-1} corresponded to the asymmetric and symmettric stretching of methylene ($-CH_2-$) group of membrane lipids, indicated that lipid in the cell treated by Osmiertinib and Kerra™ extract was slightly decreased comparing to that of control (**Fig. 4d**). Although both Osmiertinib and Kerra™ extract retarded nucleic acid production as the peak at 1238 and 1085 cm^{-1}, the secondary structure of protein accumulating in the cell were shown in different ways. Only decreasing of β-sheet was seen in the cell treated by Osmiertinib, while β-sheet slightly decreasing but higher α-helices accumulation were found in the cell treated by Kerra™ extract (**Fig. 4e** and **4f**). This evidence implies that medicinal Kerra™ extract effects the the cell is different manner from those of Osmiertinib.

Table 1. FTIR band assignments

2nd derivative peak (cm^{-1})	Band assignments
1085	Symmetric stretching vibrations of PO_2^- in nucleic acids
1238	Asymmetric stretching vibrations of PO_2^- in nucleic acids
1627	β-sheet of amide I
1650	α-helices of amide I
2921 and 2852	Asymmetric and symmetric stretching vibrations of $-CH_2-$
2960 and 2873	Asymmetric and symmetric stretching vibrations of $-CH_3$

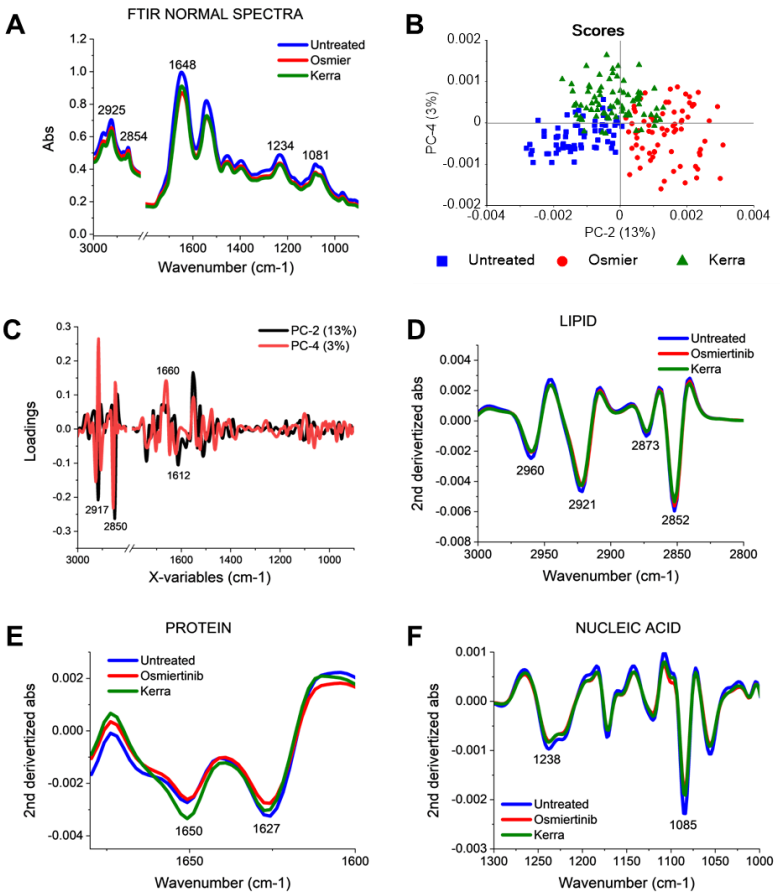


Fig. 4 FTIR spectra of A549 lung cancers. (**A**) Comparison of normal spectra of untreated, Osimertinib (4.8 µg/mL), and Kerra (386.5 µg/mL)-treated A549 within the spectral range of 3000 to 1000 cm^{-1}. (**B**) PCA score plot of untreated, Osimertinib, and Kerra-treated A549. (**C**) PCA Loading score plot of PC2 and PC4. (**D–F**) Overlaid second derivatized spectra of the lipid regions (3000 and 2800 cm^{-1}), amide regions (1740–1600 cm^{-1}), and nucleic acid (1300–1000 cm^{-1})

4. Discussion

The findings of this study highlight the potential of Kerra™ extract as an effective agent for targeting EGFR-driven lung cancer, including overcoming resistance to conventional EGFR-targeted therapies. When compared to other studies investigating novel therapeutic approaches for EGFR-positive lung cancer, Kerra™ extract offers promising results, particularly in its ability to inhibit both wild-type and drug-resistant EGFR mutants. Several studies have explored natural compounds as potential alternatives to conventional EGFR inhibitors. For example, a study by Jen-Yi Lee et al. (2011) demonstrated the anti-cancer properties of curcumin, a bioactive compound derived from turmeric, in inhibiting EGFR signaling

in lung cancer cells(27). While curcumin exhibited anti-tumor activity, its efficacy was limited by poor bioavailability. In contrast, Kerra™ extract, which appears to have a distinct molecular profile, offers an interesting alternative that may circumvent this issue. The FTIR analysis in our study suggests that Kerra™ extract induces biochemical changes similar to, but distinct from, those seen with Osimertinib, a first-line treatment for EGFR-positive lung cancer. This differentiation in molecular impact could potentially allow Kerra™ extract to target tumor cells more efficiently, even those harboring resistance mutations such as T790M and C797S, which are known to reduce the efficacy of standard EGFR inhibitors(28).

In terms of EGFR-mutant inhibition, Osimertinib has shown significant success, particularly in overcoming resistance caused by the T790M mutation(13). However, resistance to Osimertinib itself remains a major challenge, and mutations such as C797S can lead to acquired resistance to this second-generation EGFR inhibitor(8,29). Our study's findings suggest that Kerra™ extract may offer a therapeutic advantage by inhibiting both wild-type and drug-resistant EGFR variants, which could potentially make it a more versatile alternative to current treatments. This is consistent with previous studies showing that natural compounds, such as resveratrol(30,31) and quercetin(32), exhibit similar properties by modulating EGFR signaling pathways and showing efficacy against both wild-type and mutant EGFR-driven cancer cells. Another important aspect of our findings is the role of Kerra™ extract in reducing cancer cell migration, as shown in the wound healing assay. This is significant because metastasis is a major cause of poor prognosis in lung cancer patients. Research by Priyanka Chowdhury et al. (2019) demonstrated that inhibiting EGFR signaling also decreases migration and invasion in lung cancer cells(33). In our study, Kerra™ extract's ability to suppress cell migration mirrors the findings of other studies focusing on natural compounds that hinder metastasis, such as resveratrol and epigallocatechin gallate (EGCG), which have been shown to inhibit EGFR-mediated migration and invasion in various cancer cell lines(34-36). The added benefit of Kerra™ extract could be its ability to target metastasis while simultaneously inhibiting EGFR signaling, providing a dual therapeutic effect that could be crucial for improving lung cancer treatment outcomes.

Additionally, the FTIR analysis in our study revealed that Kerra™ extract induces biochemical changes that are distinct from those seen with Osimertinib. This aligns with the growing body of research that highlights the importance of investigating the molecular signatures of natural compounds. Studies like those by Atchara Paemanee et al. (2022) and Jeeraprapa Siriwaseree et al. (2022) show that plant-derived compounds can induce changes in cellular lipids, nucleic acids, and proteins, which may contribute to their anti-cancer properties(37,38). These distinct molecular changes induced by Kerra™ extract suggest that it may exert its anti-cancer effects through mechanisms that differ from conventional EGFR inhibitors, thus offering a complementary or alternative approach for EGFR-driven lung cancer. Despite these promising results, there are some limitations to the study that warrant consideration. First, the *in vitro* nature of the experiments means that further *in vivo* studies are needed to fully evaluate the therapeutic potential of Kerra™ extract in a living organism. Additionally, while our study demonstrated its ability to reduce cell viability and migration in EGFR-positive lung cancer cells, its effects on normal cells and potential side effects require further investigation to assess the overall safety and efficacy of Kerra™ extract as a treatment.

In comparison to other natural compounds tested in EGFR-driven lung cancer, Kerra™ extract holds significant promise due to its ability to target both wild-type and mutant EGFR variants, inhibit metastasis, and induce unique biochemical alterations. These findings position Kerra™ extract as a

potentially valuable addition to the arsenal of treatments for EGFR-positive lung cancer, especially in cases where resistance to traditional therapies is a challenge. However, additional research is necessary to confirm its clinical applicability and further elucidate its molecular mechanisms, which could ultimately lead to the development of more effective treatments for EGFR-mutant lung cancer.

5. Conclusion

Kerra™ extract demonstrates significant potential as an alternative or adjunctive therapy for EGFR-driven lung cancer, showing promising results in inhibiting both wild-type and mutant EGFR, as well as reducing cancer cell migration. The extract's ability to target drug-resistant EGFR mutations places it as a potential solution for overcoming resistance to current EGFR-targeted therapies like Osimertinib. While the results from this study are promising, further research, including *in vivo* studies and safety evaluations, is essential to fully assess the clinical applicability and therapeutic potential of Kerra™ extract. If further studies confirm its efficacy and safety, Kerra™ extract could become a valuable addition to the treatment options available for EGFR-mutant lung cancer, particularly in cases of resistance to conventional treatments.

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7. Author contributions

N.K. and S.S. prepared the samples and conducted the enzyme activity experiments. S.S.K. and W.T. performed the cell-based assays. B.K. performed the FTIR analysis. T.L. and B.K. analyzed the data and wrote the manuscript. T.L., B.K., and K.C. discussed the results and contributed to the final manuscript. K.C. supervised the project. All authors have read and approved the published manuscript.

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9. Conflict of Interest

The authors declare no conflict of interest.

10. References

1. Ma, M., Tan, H., Yan, H., and Zheng, K. (2024) The impact of family cancer history on tumor metabolism and prognosis in patients with non-small cell lung cancer. *Scientific Reports* **14**, 22632
2. Siegel, R. L., Miller, K. D., Wagle, N. S., and Jemal, A. (2023) Cancer statistics, 2023. *CA: A Cancer Journal for Clinicians* **73**, 17-48
3. Liu, X., Wang, P., Zhang, C., and Ma, Z. (2017) Epidermal growth factor receptor (EGFR): A rising star in the era of precision medicine of lung cancer. *Oncotarget* **8**
4. Yarden, Y., and Sliwkowski, M. X. (2001) Untangling the ErbB signalling network. *Nat Rev Mol Cell Biol* **2**, 127-137
5. Ciardiello, F., and Tortora, G. (2008) EGFR Antagonists in Cancer Treatment. *New England Journal of Medicine* **358**, 1160-1174
6. Harrison, P. T., Vyse, S., and Huang, P. H. (2020) Rare epidermal growth factor receptor (EGFR) mutations in non-small cell lung cancer. *Semin Cancer Biol* **61**, 167-179
7. Grabe, T., Lategahn, J., and Rauh, D. (2018) C797S Resistance: The Undruggable EGFR Mutation in Non-Small Cell Lung Cancer? *ACS Medicinal Chemistry Letters* **9**, 779-782
8. Rangachari, D., To, C., Shpilsky, J. E., VanderLaan, P. A., Kobayashi, S. S., Mushajiang, M., Lau, C. J., Paweletz, C. P., Oxnard, G. R., Jänne, P. A., and Costa, D. B. (2019) EGFR-Mutated Lung Cancers Resistant to Osimertinib through EGFR C797S Respond to First-Generation Reversible EGFR Inhibitors but Eventually Acquire EGFR T790M/C797S in Preclinical Models and Clinical Samples. *J Thorac Oncol* **14**, 1995-2002
9. Karachaliou, N., Fernandez-Bruno, M., Bracht, J. W. P., and Rosell, R. (2019) EGFR first- and second-generation TKIs-there is still place for them in EGFR-mutant NSCLC patients. *Transl Cancer Res* **8**, S23-s47
10. Pao, W., Miller, V. A., Politi, K. A., Riely, G. J., Somwar, R., Zakowski, M. F., Kris, M. G., and Varmus, H. (2005) Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. *PLoS Med* **2**, e73
11. Yun, C. H., Mengwasser, K. E., Toms, A. V., Woo, M. S., Greulich, H., Wong, K. K., Meyerson, M., and Eck, M. J. (2008) The T790M mutation in EGFR kinase causes drug resistance by increasing the affinity for ATP. *Proc Natl Acad Sci U S A* **105**, 2070-2075
12. Kishikawa, T., Kasai, T., Okada, M., Nakachi, I., Soda, S., Arai, R., Takigami, A., and Sata, M. (2020) Osimertinib, a third-generation EGFR tyrosine kinase inhibitor: A retrospective multicenter study of its real-world efficacy and safety in advanced/recurrent non-small cell lung carcinoma. *Thorac Cancer* **11**, 935-942
13. Jänne, P. A., Yang, J. C., Kim, D. W., Planchard, D., Ohe, Y., Ramalingam, S. S., Ahn, M. J., Kim, S. W., Su, W. C., Horn, L., Haggstrom, D., Felip, E., Kim, J. H., Frewer, P., Cantarini, M., Brown, K. H., Dickinson, P. A., Ghiorghiu, S., and Ranson, M. (2015) AZD9291 in EGFR inhibitor-resistant non-small-cell lung cancer. *N Engl J Med* **372**, 1689-1699
14. Hung, H. Y., Tseng, Y. H., Liao, C. M., Chen, S. Y., Wu, T. P., Lee, Y. C., and Chen, Y. M. (2017) The Efficacy of Traditional Chinese Herbal Medicine in the Treatment of EGFR Mutated Stage IV Pulmonary Adenocarcinoma Patients Who Received First-Line EGFR-TKI Treatment. *Integr Cancer Ther* **16**, 126-131
15. Sui, X., Zhang, M., Han, X., Zhang, R., Chen, L., Liu, Y., Xiang, Y., and Xie, T. (2020) Combination of traditional Chinese medicine and epidermal growth factor receptor tyrosine kinase inhibitors in the treatment of non-small cell lung cancer: A systematic review and meta-analysis. *Medicine* **99**, e20683
16. Lamtha, T., Tabtimmai, L., Songtawee, N., Tansakul, N., and Choowongkomon, K. (2022) Structural analysis of cannabinoids against EGFR-TK leads a novel target against EGFR-driven cell lines. *Current Research in Pharmacology and Drug Discovery* **3**, 100132
17. Seetaha, S., Khamplong, P., Wanaragthai, P., Aiebchun, T., Ratanabunyong, S., Krobthong, S., Yingchutrakul, Y., Rattanasrisomporn, J., and Choowongkomon, K. (2022) KERRA, Mixed

Medicinal Plant Extracts, Inhibits SARS-CoV-2 Targets Enzymes and Feline Coronavirus. *COVID* **2**, 621-632
18. Choowongkomon, K., Choengpanya, K., Pientong, C., Ekalaksananan, T., Talawat, S., Srathong, P., and Chuerduangphui, J. (2023) The Inhibitory Effect of Kerra(TM), KS(TM), and Minoza(TM) on Human Papillomavirus Infection and Cervical Cancer. *Medicina (Kaunas)* **59**
19. Siriwaseree, J., Yingchutrakul, Y., Samutrtai, P., Aonbangkhen, C., Srathong, P., Krobthong, S., and Choowongkomon, K. (2023) Exploring the Apoptotic-Induced Biochemical Mechanism of Traditional Thai Herb (Kerra™) Extract in HCT116 Cells Using a Label-Free Proteomics Approach. *Medicina (Kaunas)* **59**
20. Saehlee, S., Seetaha, S., Klankaew, W., Srathong, P., Choowongkomon, K., and Choengpanya, K. (2024) Anti-Human Immunodeficiency Virus-1 Property of Thai Herbal Extract Kerra™. *Pharmaceuticals (Basel)* **17**
21. Li, H., Wang, J., Zhang, G., Li, Y., Lin, L., Yang, H., Zhou, J., Zhang, L., and Lv, D. (2020) Detection of plasma T790M mutation after the first generation EGFR-TKI resistance of non-small cell lung cancer in the real world. *Journal of Thoracic Disease* **12**, 550-557
22. Fares, J., Fares, M. Y., Khachfe, H. H., Salhab, H. A., and Fares, Y. (2020) Molecular principles of metastasis: a hallmark of cancer revisited. *Signal Transduction and Targeted Therapy* **5**, 28
23. Seetaha, S., Ratanabanyong, S., and Choowongkomon, K. (2019) Expression, purification, and characterization of the native intracellular domain of human epidermal growth factor receptors 1 and 2 in Escherichia coli. *Appl Microbiol Biotechnol* **103**, 8427-8438
24. Lamtha, T., Tabtimmai, L., Bangphoomi, K., Kiriwan, D., Malik, A. A., Chaicumpa, W., van Bergen en Henegouwen, P. M. P., and Choowongkomon, K. (2021) Generation of a nanobody against HER2 tyrosine kinase using phage display library screening for HER2-positive breast cancer therapy development. *Protein Engineering, Design and Selection* **34**
25. Suwattanasophon, C., Mistlberger-Reiner, A., Alberdi-Cedeño, J., Pignitter, M., Somoza, V., König, J., Lamtha, T., Wanaragthai, P., Kiriwan, D., and Choowongkomon, K. (2023) Identification of the Brucea javanica Constituent Brusatol as a EGFR-Tyrosine Kinase Inhibitor in a Cell-Free Assay. *ACS Omega* **8**, 28543-28552
26. Jonkman, J. E., Cathcart, J. A., Xu, F., Bartolini, M. E., Amon, J. E., Stevens, K. M., and Colarusso, P. (2014) An introduction to the wound healing assay using live-cell microscopy. *Cell Adh Migr* **8**, 440-451
27. Lee, J. Y., Lee, Y. M., Chang, G. C., Yu, S. L., Hsieh, W. Y., Chen, J. J., Chen, H. W., and Yang, P. C. (2011) Curcumin induces EGFR degradation in lung adenocarcinoma and modulates p38 activation in intestine: the versatile adjuvant for gefitinib therapy. *PLoS One* **6**, e23756
28. Kobayashi, S., Boggon, T. J., Dayaram, T., Jänne, P. A., Kocher, O., Meyerson, M., Johnson, B. E., Eck, M. J., Tenen, D. G., and Halmos, B. (2005) EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. *N Engl J Med* **352**, 786-792
29. Planchard, D., Smit, E. F., Groen, H. J. M., Mazieres, J., Besse, B., Helland, Å., Giannone, V., D'Amelio, A. M., Jr., Zhang, P., Mookerjee, B., and Johnson, B. E. (2017) Dabrafenib plus trametinib in patients with previously untreated BRAF(V600E)-mutant metastatic non-small-cell lung cancer: an open-label, phase 2 trial. *Lancet Oncol* **18**, 1307-1316
30. Wang, Y., Romigh, T., He, X., Orloff, M. S., Silverman, R. H., Heston, W. D., and Eng, C. (2010) Resveratrol regulates the PTEN/AKT pathway through androgen receptor-dependent and -independent mechanisms in prostate cancer cell lines. *Human Molecular Genetics* **19**, 4319-4329
31. Jin, Z., Feng, W., Ji, Y., and Jin, L. (2017) Resveratrol mediates cell cycle arrest and cell death in human esophageal squamous cell carcinoma by directly targeting the EGFR signaling pathway. *Oncol Lett* **13**, 347-355
32. Chen, X., Xu, P., Zhang, H., Su, X., Guo, L., Zhou, X., Wang, J., Huang, P., Zhang, Q., and Sun, R. (2021) EGFR and ERK activation resists flavonoid quercetin-induced anticancer activities in human cervical cancer cells in vitro. *Oncol Lett* **22**, 754

33. Chowdhury, P., Dey, P., Ghosh, S., Sarma, A., and Ghosh, U. (2019) Reduction of metastatic potential by inhibiting EGFR/Akt/p38/ERK signaling pathway and epithelial-mesenchymal transition after carbon ion exposure is potentiated by PARP-1 inhibition in non-small-cell lung cancer. *BMC Cancer* **19**, 829
34. Zhao, Y., Tang, H., Zeng, X., Ye, D., and Liu, J. (2018) Resveratrol inhibits proliferation, migration and invasion via Akt and ERK1/2 signaling pathways in renal cell carcinoma cells. *Biomedicine & Pharmacotherapy* **98**, 36-44
35. Zhu, W., Li, M. C., Wang, F. R., Mackenzie, G. G., and Oteiza, P. I. (2020) The inhibitory effect of ECG and EGCG dimeric procyanidins on colorectal cancer cells growth is associated with their actions at lipid rafts and the inhibition of the epidermal growth factor receptor signaling. *Biochemical Pharmacology* **175**, 113923
36. Minnelli, C., Cianfruglia, L., Laudadio, E., Mobbili, G., Galeazzi, R., and Armeni, T. (2021) Effect of Epigallocatechin-3-Gallate on EGFR Signaling and Migration in Non-Small Cell Lung Cancer. *Int J Mol Sci* **22**
37. Paemanee, A., Rattanabunyong, S., Ketngamkum, Y., Siriwaseree, J., Pongpamorn, P., Romyanon, K., Tangphatsornruang, S., Kuaprasert, B., and Choowongkomon, K. (2022) Mass spectrometry and synchrotron-FTIR microspectroscopy reveal the anti-inflammatory activity of Bua Bok extracts. *Phytochemical Analysis* **33**, 1086-1098
38. Siriwaseree, J., Sanachai, K., Aiebchun, T., Tabtimmai, L., Kuaprasert, B., and Choowongkomon, K. (2022) Synchrotron Fourier Transform Infrared Microscopy Spectra in Cellular Effects of Janus Kinase Inhibitors on Myelofibrosis Cancer Cells. *ACS Omega* **7**, 22797-22803

figure 1

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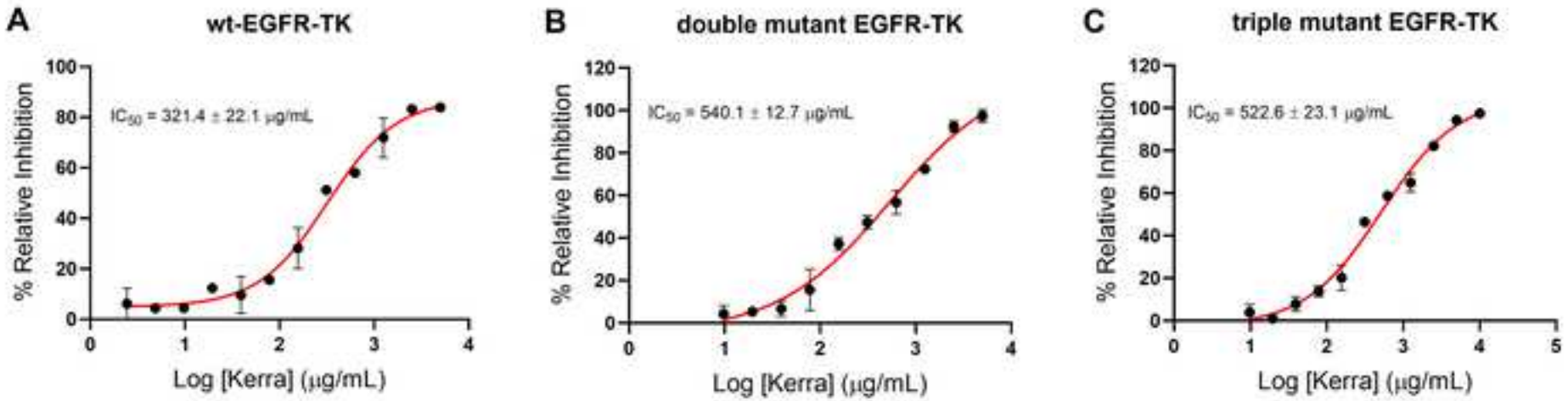


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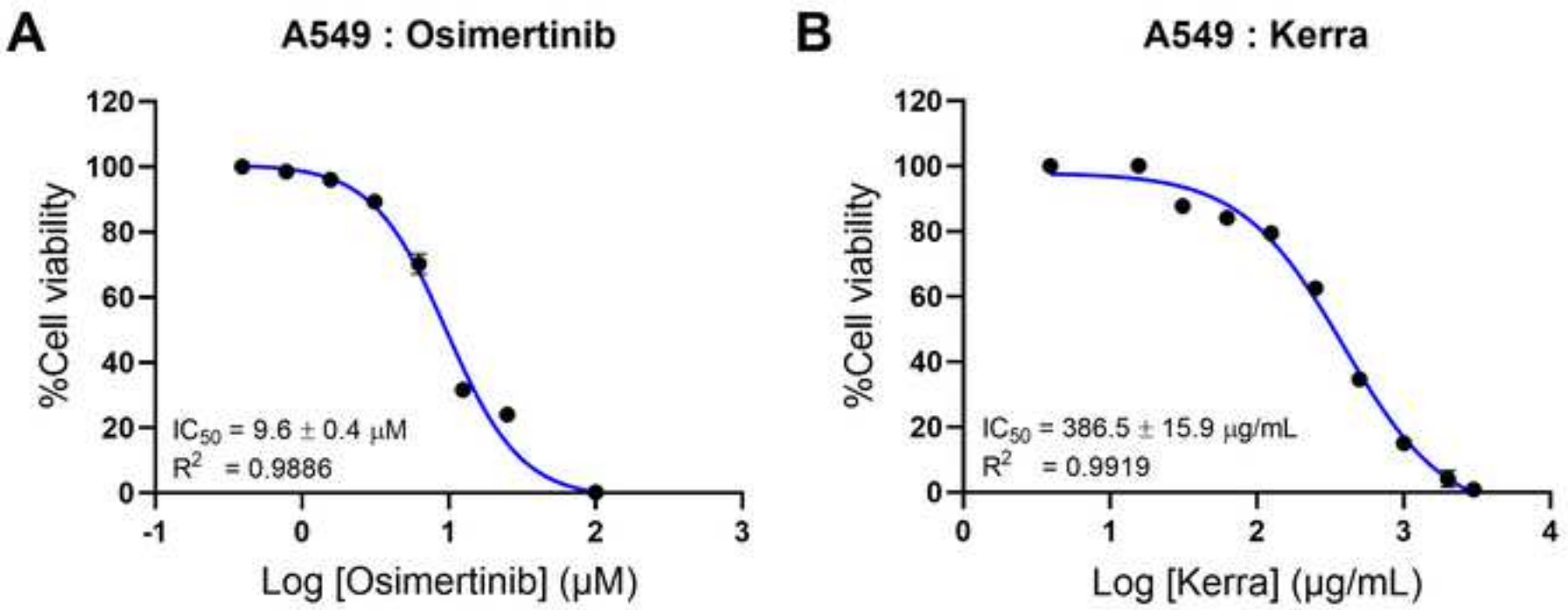


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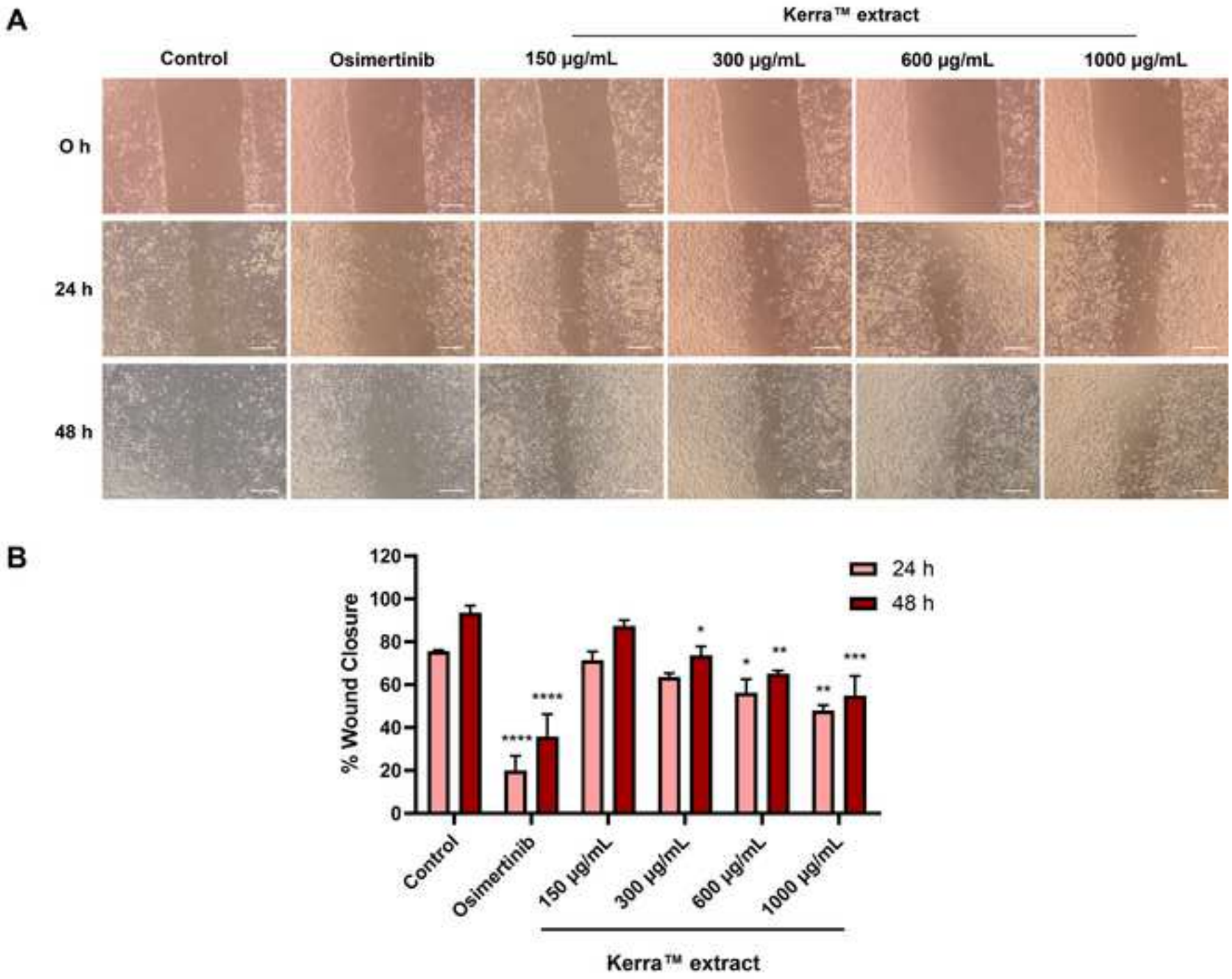
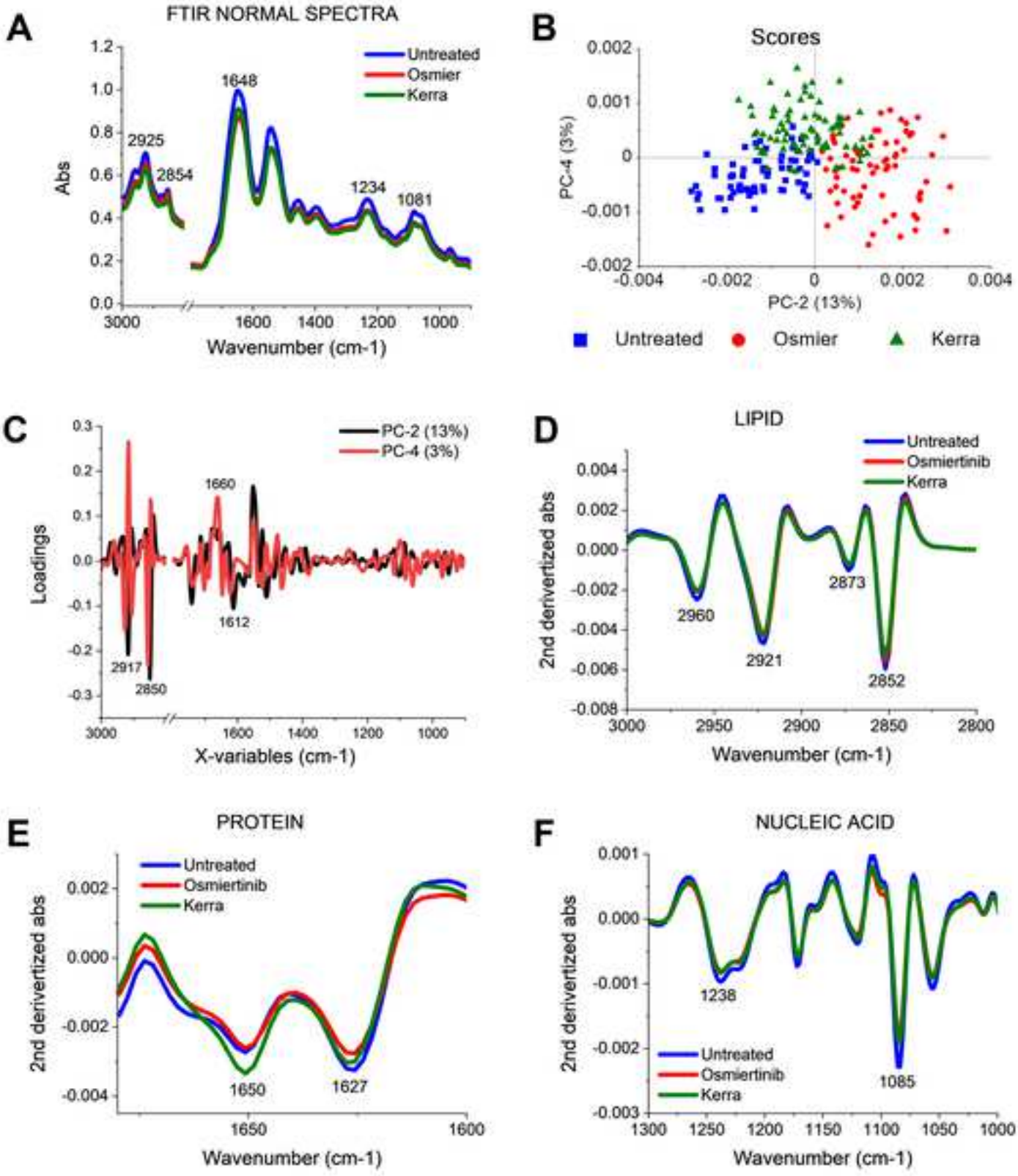


figure 4

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