

Antioxidant, Anti-Inflammatory, and Anticancer Activities of Ethanolic Chayote (*Sechium edule*) Fruit Extract: Phytochemical Insights and Therapeutic Implications

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ABSTRACT

Chayote (*Sechium edule*) is a valuable species of the *Cucurbitaceae* family, widely consumed in many countries due to its diverse nutritional and bio-functional properties. This study was designed to evaluate the phenolic compounds (using Folin-Ciocalteu and aluminum chloride methods), antioxidant (via DPPH assay), anti-inflammatory, and anti-cancer activity (by MTT assay) of chayote fruit ethanolic extract (CFE). The results showed that the CFE contains high phenolic and flavonoid compounds with concentration-dependent and appropriate antioxidant activity. Approximately 62 ± 0.53 % of DPPH free radicals were inhibited at the $200 \mu\text{g mL}^{-1}$ concentration ($\text{IC}_{50} = 35.40 \mu\text{g mL}^{-1}$). Also, the results of the anti-inflammatory activity indicated that CFE significantly prevents protein denaturation. The highest inhibitory effect at $800 \mu\text{g mL}^{-1}$ was 63.37 ± 2.14 %. Further, the CFE anticancer activity results on breast cancer cell lines (MCF-7) indicated its significant toxicity ($\text{IC}_{50} = 42.04 \mu\text{g mL}^{-1}$) after 24 h. However, no significant toxicity was observed in normal mouse fibroblast cells (L929). Generally, this study demonstrates that CFE is rich in bioactive compounds with antioxidant, anti-inflammatory, and anticancer effects, making it a promising candidate for use in functional foods and pharmaceutical applications.

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1. Introduction

Utilizing herbal plants as one of the richest and most recognized resources in novel treatment methods represents a dynamic intersection of traditional knowledge and modern science (Fazeli-Nasab and Mirzaei, 2018). Their easy accessibility, high biocompatibility, and low cost are the primary reasons for the growing interest in plant-based therapeutic approaches. Recent studies have increasingly highlighted using plant essential oils and extracts as natural agents with significant biological properties for food preservation and the development of novel therapeutic approaches. As research continues to validate and explore the therapeutic potential of these plants, they are likely to play an increasingly significant role in contemporary healthcare practices (Chen and Dou, 2008; Najmi *et al.*, 2022; Napier *et al.*, 2023). Chayote (*Sechium edule* (Jacq.)) is a herbaceous

and perennial plant of the *Cucurbitaceae* family with tuberous roots native to South America and Oceania. Chayote exhibits resistance to the majority of common diseases and pest infestations, including those caused by fungi, nematodes, and insects (Vieira *et al.*, 2019). Studies suggest that compounds found in Chayote, such as flavonoids and alkaloids, may possess anti-inflammatory and diuretic properties while aiding in blood sugar regulation (Rosado-Pérez *et al.*, 2019). Further, its low-calorie and high-fiber composition makes it a great option for promoting digestive wellness and effectively managing weight. The fruit of this plant is highly valued for consumption, commonly prepared using standard cooking methods or incorporated into stews and desserts. In contrast, the skin and leaves are typically utilized in smaller quantities, yet they exhibit promising therapeutic properties (Loizzo *et al.*, 2016).

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Chayote fruit extract contains abundant bioactive compounds, including phenolic compounds, peroxidases, alkaloids, flavonoids, carotenoids, saponins, and phytosterols. These compounds exhibit potential therapeutic benefits, including antimicrobial, anticancer, anti-inflammatory, and antihypertensive effects (Aguñiga-Sánchez *et al.*, 2020). Studies have demonstrated that chayote extract can inhibit the proliferation of tumor cell lines while preserving the integrity of normal cells (Aguñiga-Sánchez *et al.*, 2017). Research has also indicated that the extract from the edible species of this plant contains active compounds with high antioxidant properties, capable of preventing the production of free radicals and shielding cells against oxidative damage (Rosado-Pérez *et al.*, 2018). Utilizing these natural antioxidants in food products can reduce reliance on synthetic preservatives. Moreover, the anti-inflammatory properties of the plant's extracts indicate that it may help modulate inflammatory pathways, potentially reducing the risk of chronic diseases. However, research and development are necessary to optimize the methods of extraction and application of these compounds and ensure the maintenance of their effectiveness in food products. The extraction method and solvent choice are critical in determining the biological activity of chayote fruit extract. Utilizing advanced extraction techniques and selecting appropriate solvents can significantly enhance the yield and efficacy of bioactive compounds, thereby maximizing the health benefits associated with chayote. This understanding is essential for researchers and industries looking to harness the full potential of this nutritious fruit (Hayouni *et al.*, 2007). Ultimately, this strategy may result in innovative formulations that enhance food safety and contribute to public health, effectively addressing the dual challenges of chronic disease prevention and food sustainability (Singh *et al.*, 2022). Accordingly, this study evaluates the phenolic and flavonoid content of chayote fruit ethanolic extract (CFE) and hypothesizes that its antioxidant, anti-inflammatory, and anti-cancer activities will support its application in functional foods and pharmaceuticals.

2. Materials and methods

2.1. Materials

Dimethyl sulfoxide (DMSO), fetal bovine serum (FBS), 3-(4,5-dimethylthiazol-2-yl)-2,5-

diphenyltetrazolium bromide (MTT), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and bovine serum albumin (BSA) were acquired from Merck, Germany. Mouse fibroblast cells (L-929 line) and breast cancer cells (MCF-7 line) were obtained from the Pasteur Institute of Iran. Aluminum chloride, ethanol, and sodium carbonate were purchased from Scharlau, Spain.

2.2. Preparation of chayote fruit ethanolic extract

The chayote (*Sechium edule*) fruits were collected from the heights of the Bandpay region in Babol, Mazandaran province, Iran. Then, the fruits were dried at 40°C and thoroughly ground into powder using a mill. Subsequently, 1 g of this powder was weighed and mixed with 70% ethanol (20 mL). The mixture was stirred continuously at 120 rpm for 24 h. After filtration through Whatman No. 1 filter paper, the solvent was evaporated from the extract via a rotary evaporator (RV 10 Digital V_C, made in Germany). Finally, the chayote fruit ethanolic extract (CFE) was dried using a freeze dryer (OPERON, made in South Korea) at -40°C and stored at 4°C (Ghanbari Hassan Kiadeh *et al.*, 2021).

2.3. Quantification of total phenolic content

The total phenolic content of the CFE was measured using the Folin-Ciocalteu assay. Briefly, 50 µL of Folin-Ciocalteu (1M) was mixed with 100 µL of CFE (1 mg mL⁻¹) and then homogenized with 1.85 mL of deionized (DI) water at room temperature (RT). Afterward, 300 µL of 20% (w/v) sodium carbonate solution was added to the mixture, ensuring it was well combined. After 2 min, 1.7 mL of DI water was added, and the solution was stirred and placed in the dark for 90 min. Finally, the samples' absorbance was measured at 760 nm using a UV-Vis spectrophotometer, and the measurements were quantified and reported as milligrams (mg) gallic acid equivalents per gram of sample in dry weight (GAE g⁻¹ DW). The gallic acid standard curve was also established at concentrations ranging from 1 to 200 µg mL⁻¹ (Alipour Kakroudi *et al.*, 2021).

2.4. Quantification of total flavonoid content

The aluminum chloride colorimetric assay quantified the total flavonoid content of CFE. For this purpose, 1500 µL of the CFE (1 mg mL⁻¹), an equal volume of aluminum chloride solution (20%), was added and stirred at RT for 40 min in the dark

conditions. The solution's absorbance was evaluated at a wavelength of 415 nm. Finally, the total flavonoid content was reported as mg quercetin per gram of sample in dry weight (mg QE g⁻¹ DW). The quercetin standard curve was also prepared at various concentrations ranging from 1 to 200 µg mL⁻¹ (Govahi et al., 2021).

2.5. Antioxidant activity of CFE using the DPPH method

To evaluate the antioxidant properties, 1 mL of CFE (5, 10, 25, 50, 100, and 200 µg mL⁻¹) was mixed with 1 mL of methanolic DPPH solution (50 µM). The final volume was adjusted to 4 mL with methanol, and the mixture was kept at RT for 30 min in the dark. Methanol was used as a blank, DPPH solution as a control, and ascorbic acid as a standard (positive controls). Finally, the absorbance of the samples was measured at a wavelength of 517 nm (Ghasemi et al., 2023). The DPPH free radical scavenging percentage (%) was determined by the following equation (Equation 1):

$$(1) \quad \text{DPPH free radical scavenging (\%)} = \frac{A_D - A_S}{A_D} \times 100$$

A_D is the control absorbance (DPPH solution), while A_S is the CFE absorbance.

2.6. Anti-inflammatory activity

The CFE's anti-inflammatory was evaluated based on its capacity to inhibit the denaturation of bovine serum albumin (BSA) protein, via a modified version of the method described by Sharifi et al. (2020). Briefly, 2 mL of CFE (25, 50, 100, 200, 400, and 800 µg mL⁻¹) was combined with phosphate-buffered saline solution (2.8 mL, pH 6.6) and 0.2 mL of 1% (w/v) BSA standard solution. The mixture was incubated at 37°C for 20 min and then heated at 75°C for 5 min. The BSA standard solution was considered the control, and diclofenac sodium (10 to 100 µg mL⁻¹) was used as the standard drug (Sharifi-Rad et al., 2020). The turbidity of the samples was measured at 660 nm via a UV-Vis spectrophotometer. Finally, the protein denaturation inhibition (%) was calculated using the following equation (Equation 2):

$$(2) \quad \text{Inhibition (\%)} = 100 \times \left[1 - \left(\frac{A_S}{A_C} \right) \right]$$

where A_S is the sample absorption and A_C is the control absorption.

2.7. Determination of cytotoxic activity by MTT assay

Cancer cell metabolic activity following CFE treatment was assessed through MTT assays. Initially, the breast cancer cells (MCF-7) and normal mouse fibroblast cell line (L929) were grown in DMEM medium with 10% fetal bovine serum (FBS) and maintained at standard conditions (37°C and 5% CO₂). When the cells had achieved at least 80% confluency, they were dislodged from the flask with trypsin-EDTA and centrifuged for 5 min. Then, the cell suspension was transferred to a cell culture plate 96 well. After 24 h of incubation, the culture medium was carefully aspirated, and the CFE at specified concentrations (2, 4, 8, 16, 32, and 64 µg mL⁻¹) was added to the wells. After 48 h, the supernatant was removed, and 200 µL of MTT solution was added to the wells in the dark. Then, the plate was incubated at 37°C for 4 h. Eventually, the MTT-containing medium was taken out, and DMSO (200 µL) was introduced to dissolve the formazan crystals (Ghanbari Hassan Kiadeh et al., 2024). Finally, the optical density (OD) of each sample was determined at 570 nm using an ELISA reader (BioTek ELX800, USA). Untreated cells were considered the control. Also, the cell viability was assessed via the following equation (Equation 3):

$$(3) \quad \text{Cell viability (\%)} = \frac{\text{OD of treated cells}}{\text{OD of Control cells}} \times 100$$

2.8. Statistical analysis

The study findings were reported as mean ± standard deviation (Mean ± SD) and compared using a one-way analysis of variance (ANOVA) study, followed by Duncan's test through a completely randomized design. A significance level of P-value <0.05 was used for statistical evaluation. The data analyses were conducted with the SPSS 26 software.

3. Results and discussion

3.1. Determination of phenolic compounds

The CFE's total phenol and flavonoid contents were determined by *in vitro* assays. Phenolic compounds are a group of aromatic secondary metabolites in plants that are very important in the medicine, pharmaceutical, and food industries due to their

positive biological effects, such as antioxidant and anticancer activity (Lakshmanashetty *et al.*, 2010). According to the obtained results, the total phenol and flavonoid contents were measured as 19.8 ± 0.82 mg GAE g^{-1} DW and 20.25 ± 1.69 mg QE g^{-1} DW, respectively. Until now, numerous studies have examined the content of phenolic compounds in chayote fruit extract. However, it has been found that the type and content of the secondary metabolites of the extract largely depend on the studied species and variety, which has repercussions on its biological activities (Salazar-Aguilar *et al.*, 2017). Geographical location, climate, harvest season, and soil type can also affect the phenolic compounds content in plants (Raeisi *et al.*, 2016). It also seems that utilizing various parts of the plant and the type of solvent used can be important determining factors in the amount of these compounds. Fidrianny *et al.* (2015) in a comparative study evaluating the bioactive compounds of different parts of chayote reported the highest total phenolic content in the ethyl acetate fruit extract, about 30.21 mg GAE g^{-1} DW. The highest total flavonoid content was recorded in the ethyl acetate leaf extract (Fidrianny *et al.*, 2015). They also stated that phenolic and flavonoid compounds were the main contributors to the antioxidant capacity of chayote fruit and stem extracts.

3.2. DPPH free radical scavenging assay

CFE's antioxidant properties were analyzed through the DPPH free-radical scavenging assay. The existence of free radicals in the body may result in a range of health issues, such as cancer, diabetes, heart disease, neurological impairment, muscular problems, premature aging, vision impairment, and a compromised immune system. Antioxidants are the main way to fight free radicals and regenerate damaged cells (Lorenzo *et al.*, 2018). The results indicated that the CFE's scavenging activity was concentration-dependent. The highest free radical scavenging rate was $62 \pm 0.53\%$ at $200 \mu g mL^{-1}$, and the IC_{50} value was also recorded at $35.40 \pm 0.82 \mu g mL^{-1}$. Ascorbic acid, as a positive control, demonstrated an IC_{50} value equal to $26 \pm 0.14 \mu g mL^{-1}$. These results indicate that although the antioxidant activity of CFE is lower than that of the positive control (p -value < 0.05), it significantly reduces the value of DPPH free radicals. This can be attributed to the activity of the phenolic and flavonoid compounds of the extract. Studies have

shown that as the concentration of phenolic compounds rises, increasing the number of hydroxyl groups leads to a higher likelihood of hydrogen bonding with free radicals, enhancing the extract's antioxidant properties (Zhang *et al.*, 2009). As a potent repository of bioactive antioxidants, chayote extract can help reduce oxidative stress in the body. In recent years, natural antioxidants have found various uses as preservatives in the food and cosmetics industries. Therefore, finding novel sources with proper antioxidant properties will be important (Oswell *et al.*, 2018). The scavenging percentage of DPPH radicals by different concentrations of CFE is shown in Fig. 1.

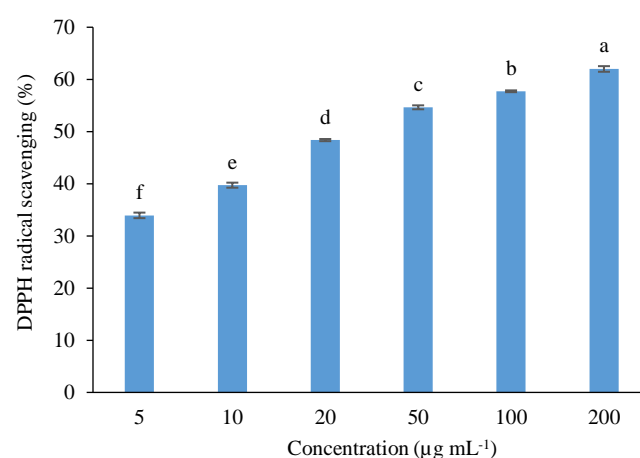


Figure 1. DPPH radical scavenging activity of CFE. (Columns with different letters indicate significant differences at P -value < 0.05).

3.3. Anti-inflammatory effect

A protein denaturation inhibition model was employed to assess CFE's anti-inflammatory potential. This method is widely used to investigate the anti-inflammatory activity of various substances, particularly in the context of natural products and pharmaceuticals. In this way, the temperature leads to the denaturation of proteins and the formation of turbidity (cloudiness), which can be measured using a spectrophotometer (Chaiya *et al.*, 2022). The results of this test showed significant anti-inflammatory activity of the extract in a dose-dependent manner. The highest inhibitory effect was observed at a concentration of $800 \mu g mL^{-1}$, with an inhibition of about $63.37 \pm 2.14\%$. The IC_{50} value was also recorded at $223.16 \pm 20.57 \mu g mL^{-1}$. This activity is mainly attributed to antioxidant compounds such as phenolic acids, tannins, and flavonoids (Vieira *et al.*, 2019). Diclofenac sodium, as the standard drug, demonstrated an IC_{50} value equal to $41.46 \pm 1.20 \mu g mL^{-1}$. The existence of such a

significant (p -value <0.05) difference could be due to the difference in the purity percentage of the extract and the standard drug. Herbal extracts are a complex mixture, only a part of which consists of bioactive compounds, and a major part of which may include sugars, proteins, fibers, and other inactive substances (Heinrich et al., 2022). However, these results can be a starting point for purification, identification of the main active compound, and synthesis of more potent derivatives. Research indicates that the plant extract-derived secondary metabolites can suppress the release of lysosomal enzymes from neutrophils at the site of inflammation. These enzymes, which include proteinases and antibacterial agents, are known to provoke inflammation and harm tissues (Sharifi-Rad et al., 2020). These effects can be especially beneficial for people suffering from chronic inflammatory diseases such as metabolic syndrome (MetS). Gavia-García et al. (2023) found that chayote supplementation has anti-inflammatory and protective effects against telomere shortening in older adults with MetS. The anti-inflammatory effect of different concentrations of CFE is shown in Fig. 2.

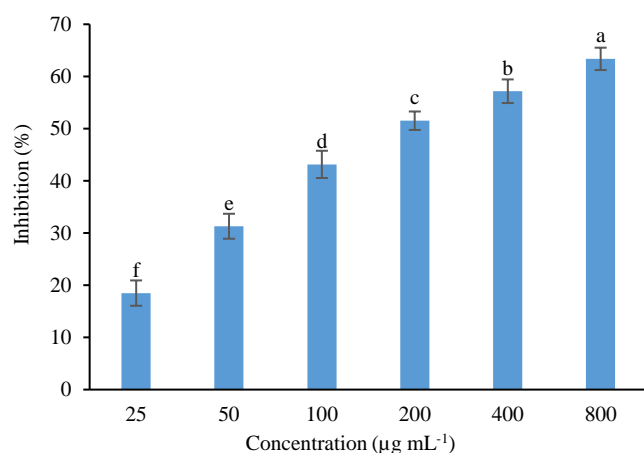


Figure 2. The anti-inflammatory effect of CFE. (Columns with different letters indicate significant differences at P -value <0.05).

3.4. In vitro cytotoxic activity by MTT assay

The cytotoxicity of CFE was evaluated in breast cancer cell lines (MCF-7) and normal mouse fibroblasts (L929) using an MTT assay. Viable cells have active mitochondria that can convert the yellow MTT dye into an insoluble purple product called formazan. The quantity of this product generated correlates directly with the number of living cells, enabling scientists to assess cell viability by measuring the extent of color transformation (Patravale et al.,

2012). The MTT assay results indicated that CFE has significant and dose-dependent anticancer efficacy against the MCF-7 cancer cell line. At the highest concentration ($64 \mu\text{g mL}^{-1}$), $54.97 \pm 2\%$ of cancer cells were destroyed. Also, the IC_{50} obtained after 24 h of treatment was recorded as $42.04 \mu\text{g mL}^{-1}$. Meanwhile, different concentrations of the CFE did not have significant cytotoxicity on the L929 cell line. After 24 h, about $82.61 \pm 1.26\%$ of normal cells treated with the highest concentration ($64 \mu\text{g mL}^{-1}$) survived. This can indicate the appropriate biocompatibility of the CFE.

The review of past studies shows that until now, there has not been a detailed evaluation of the anticancer activity of CFE on MCF-7 cell lines. Elavarasan et al. (2017) explored the photocatalytic, antibacterial, and cytotoxic properties of ZnO nanoparticles synthesized using chayote leaf extract. This study revealed that these nanoparticles exhibited notable cytotoxicity against MCF-7 cell lines ($\text{IC}_{50} = 3.5 \mu\text{g mL}^{-1}$) (Elavarasan et al., 2017). Also, Salazar et al. (2017) stated that the methanolic extract of chayote fruit can significantly prevent the growth and proliferation of the human cervical cancer HeLa cell line. Some studies indicate that these extracts can prevent the cancer cells' proliferation through apoptosis, although the specific mechanisms are still being investigated. CFE may also inhibit cancer cell migration and invasion (metastasis) (Rivera-Martínez et al., 2023). These mechanisms can highlight the potential of this medicinal supplement in cancer prevention and treatment. Fig. 3 and 4 demonstrate the anticancer activity of different concentrations of CFE on MCF-7 and L929 cell lines.

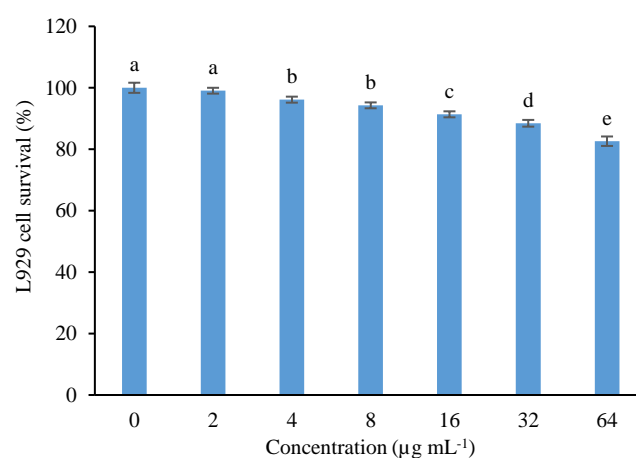


Figure 3. In vitro cytotoxicity activity of the CFE on L929 cell lines after 24 h. (Columns with different letters indicate significant differences at P -value <0.05).

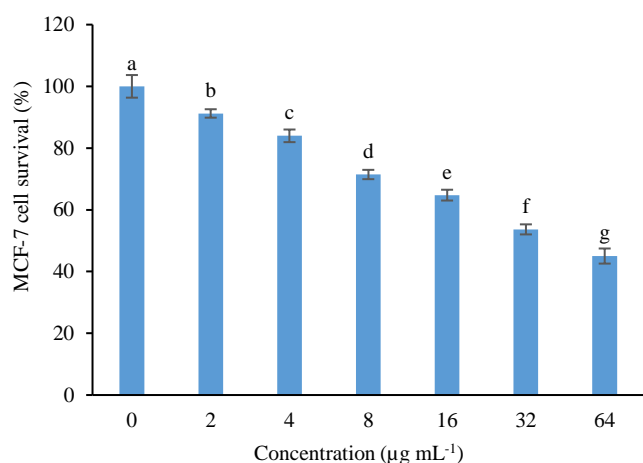


Figure 4. *In vitro* cytotoxicity activity of the CFE on MCF-7 cell lines after 24 h. (Columns with different letters indicate significant differences at P-value <0.05).

4. Conclusion

Herbal extracts offer a variety of advantages, such as being natural and potentially having fewer side effects compared to synthetic alternatives. The principal objective of this investigation was to assess the antioxidant, anti-inflammatory, and anticancer activity of chayote fruit ethanolic extract (CFE). The results showed that CFE has antioxidant and anti-inflammatory properties in a dose-dependent manner with appropriate phenolic and flavonoid compounds. It was also found that CFE has a promising therapeutic potential due to its proper anticancer properties on MCF-7 cell lines and the lack of significant toxicity on normal cells. These findings depicted CFE as a compelling natural candidate for the development of nutraceuticals, preventive health supplements, and adjunctive therapies. However, further research and clinical trials may be warranted to explore the full extent of its benefits and potential applications in healthcare and medicine. Future studies could focus on validating these findings through *in vivo* models to assess their bioavailability and efficacy. Furthermore, the isolation of bioactive compounds associated with the observed effects will be crucial for drug development, standardization, and a better understanding of mechanisms.

Conflict of interests

All authors declare no conflict of interest.

Ethics approval and consent to participate

No humans or animals were used in the present research. The authors have adhered to ethical

standards, including avoiding plagiarism, data fabrication, and double publication.

Consent for publications

All authors read and approved the final manuscript for publication.

Availability of data and material

All the data are embedded in the manuscript.

Authors' contributions

S.R. conceptualized the research, administered the project, interpreted data, and contributed to the writing/editing of the manuscript. S.G.H.K. wrote the main manuscript text, prepared figures, conducted biological experiments, performed statistical analysis, and interpreted results. M.G. conducted biological experiments and performed statistical analysis. M.G.H. conducted biological experiments and contributed to writing the manuscript and interpreting results.

Informed consent

The authors declare not to use any patients in this Research.

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