

## Citrus Limon Has the Potential to Reduce Oxidative Stress and Inflammation After Physical Activity/Exercise: Systematic Review

### Citrus Limon tiene el potencial de reducir el estrés oxidativo y la inflamación después de la actividad física/ejercicio: revisión sistemática

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**Abstract.** This study aims to analyze and highlight the potential of citrus limon in reducing inflammation and oxidative stress after physical activity/exercise. This study uses a systematic review method by searching various journal databases such as Scopus, Web of Science, Pubmed, and Embase. The inclusion criteria in this study were articles published within the last 5 years and articles discussing Limon, Inflammation, Free Radicals, and physical exercise. Exclusion criteria in this study were articles published in disreputable journals. A total of 1665 articles from Scopus, Web of Science Pubmed and Embase databases were identified. A total of 7 articles that met the inclusion criteria were selected and analyzed for this systematic review. For standard operations, this study follows the Preferred Reporting Items for Systematic review and Meta-Analyses (PRISMA) assessment. The results of this systematic research review report that the flavanoid content found in citrus limon has anti-oxidant properties that can reduce oxidative stress. Furthermore, the anti-inflammatory properties of citrus limon can reduce uncontrolled inflammation due to physical activity and intense exercise. In this case, citrus limon works by inhibiting inflammation through NF-kB signaling and reducing inflammation by suppressing the secretion of pro-inflammatory cytokines such as TNF- $\alpha$ . Reducing inflammation can potentially reduce the intensity of muscle pain. We recommend that citrus limon be used in individuals to reduce oxidative stress and inflammation caused by physical activity and intense exercise.

**Keywords:** Citrus Limon; Oxidative Stress; Inflammation; Physical training; Healthy Lifestyle

**Resumen.** Este estudio tiene como objetivo analizar y resaltar el potencial del limón cítrico para reducir la inflamación y el estrés oxidativo después de la actividad/ejercicio físico. Este estudio utiliza un método de revisión sistemática mediante la búsqueda en varias bases de datos de revistas como Scopus, Web of Science, Pubmed y Embase. Los criterios de inclusión en este estudio fueron artículos publicados en los últimos 5 años y artículos sobre Limón, Inflamación, Radicales Libres y ejercicio físico. Los criterios de exclusión en este estudio fueron artículos publicados en revistas de mala reputación. Se identificaron un total de 1665 artículos de las bases de datos Scopus, Web of Science Pubmed y Embase. Para esta revisión sistemática se seleccionaron y analizaron un total de 7 artículos que cumplieron con los criterios de inclusión. Para operaciones estándar, este estudio sigue la evaluación de elementos de informe preferidos para revisión sistemática y metanálisis (PRISMA). Los resultados de esta revisión sistemática de la investigación informan que el contenido de flavonoides que se encuentra en el limón cítrico tiene propiedades antioxidantes que pueden reducir el estrés oxidativo. Además, las propiedades antiinflamatorias del limón cítrico pueden reducir la inflamación incontrolada debida a la actividad física y al ejercicio intenso. En este caso, el limón cítrico actúa inhibiendo la inflamación a través de la señalización de NF-kB y reduciendo la inflamación al suprimir la secreción de citoquinas proinflamatorias como el TNF- $\alpha$ . Reducir la inflamación puede potencialmente reducir la intensidad del dolor muscular. Recomendamos el uso del limón cítrico en individuos para reducir el estrés oxidativo y la inflamación causada por la actividad física y el ejercicio intenso.

**Palabras clave:** Cítrico Limón; Estrés Oxidativo; Inflamación; Entrenamiento físico; Estilo de vida saludable

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## Introduction

Regular exercise can improve health and fitness (Rueggesser & Booth, 2018). But on the other hand, intense physical activity and exercise will trigger uncontrolled oxidative stress due to an imbalance between reactive oxygen species (ROS) and antioxidants (Thirupathi et al., 2021). Some literature explains that malondialdehyde (MDA) and protein carboline (PC) are biomarkers that indicate oxidative stress (El Assar et al., 2022). Increased ROS can cause degenerative diseases such as cancer, cell damage, and type 1 diabetes (Darenskaya et al., 2021). Apart from that, exercises that are done intensely also result in delayed onset muscle soreness (DOMS) (Sonkodi, 2021). Muscle pain is caused by an increase in cytokines during the inflammatory process (Ayubi et al., 2022). Several studies

have reported that TNF- $\alpha$  is a pro-inflammatory cytokine that triggers muscle pain (Ayubi et al., 2022; Fernández-Lázaro et al., 2020; Nanavati et al., 2022).

In theory, muscle pain reaches its peak within 24 to 48 hours after exercise (Anugrah et al., 2023; Chang et al., 2021; Hung et al., 2021). The phenomenon in the world today is that as many as 30 million people manage pain using non-steroidal anti-inflammatory drugs (NSAIDs) which are certainly not good for health (Kafrawi et al., 2023). In addition, a recent survey reported that as many as 17 million people in countries around the world died from degenerative diseases (Ayubi, Yuniarti, et al., 2022).

Alternative solutions are very important to look for in overcoming these problems. One of the common natural ingredients we find in the market is citrus limon. Research has reported that citrus limon has been widely used as an

ingredient in beauty products (Klimek-Szczykutowicz et al., 2020). Apart from that, citrus limon is also well-known as a natural ingredient for diet programs for weight loss (Magurano et al., 2021). In the medical world, citrus limon have been reported to treat sore throats due to viral infections (Magurano et al., 2021). In this case, the many benefits of citrus limon give us the opportunity to relate and discuss in depth the effect of limon in reducing uncontrolled oxidative stress and inflammation after physical activity through a systematic review.

This study aims to analyze and highlight the potential of citrus limon in reducing inflammation and oxydatif stress after physical activity/exercise.

## Methods

This study uses a systematic review method by searching various journal databases such as Scopus, Web of Science, Pubmed and Embase. The inclusion criteria in this study were articles published within the last 5 years and articles discussing Citrus Limon, Inflammation, Oxydatif Stress, and Physical Exercise. Exclusion criteria in this study were

articles published in disreputable journals. The title, abstract and full text of the article are filtered and then verified and stored in the Mendeley software. A total of 1665 articles from Scopus, Web of Science Pubmed and Embase databases were identified. A total of 7 articles that met the inclusion criteria were selected and analyzed for this systematic review. For standard operations, this study follows the Preferred Reporting Items for Systematic review and Meta-Analyses (PRISMA) assessment.

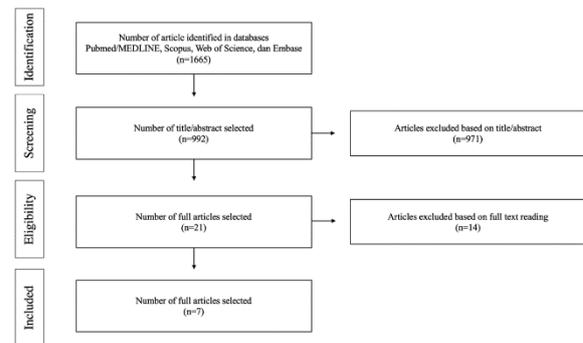


Figure 1. PRISMA flowchart of the article selection process

## Results

Table 1.  
Results of a review of the effects of limon on oxidative stress and inflammation

Author	Sample Characteristics	Study Design	Intervention	Results
(Buchwald-Werner et al., 2018)	19 healthy men and 21 women aged 22-50 years were involved in this study. Subjects were divided into two groups, namely the group with the intervention of supplementation of citrus limon extract and the group with the placebo intervention.	Experimental	Supplementation of citrus limon extract with a dose of 400 mg. intervention was given once a day. The intervention was carried out for 15 days (10 days before intensive training, one day during the test, and 4 days after.	Citrus limon extract supplementation can reduce pain intensity and prevent a decrease in muscle strength due to muscle damage and post-exercise inflammatory processes.
(Norouzi et al., 2020)	40 male mice weighing 40 grams were randomly divided into four groups: the control group, the swimming group, the limon essential oil group and the swimming group with limon essential oil.	Experimental	Swimming training was carried out for 4 weeks with a duration of 30 minutes each session. In a week, five sessions were carried out. The dose of limon essential oil was given at a dose of 50 mg/kg.	Swimming exercise with limon essential oil can reduce triglycerides and MDA levels.
(Yang et al., 2023)	A total of 21 types of citrus cultivars were extracted by hydrodistillation using a Clevenger type apparatus.	Laboratory experiment	The chemical composition of the citrus extracts was analyzed	Components in citrus limon have anti-inflammatory properties so they can potentially reduce inflammation.
(Harahap et al., 2023)	Boxing athletes aged 20-22 years were enrolled in this study. Subjects were divided into three groups, namely the gymnastics + no massage group, the gymnastics + massage with topical oil group, and the gymnastics + massage with citrus limon oil group.	Experimental	Exercise is done 3 times a week for 4 weeks. Interventions are given according to each group. Massage is done after 30 minutes after training.	Citrus limon used as massage therapy oil can reduce muscle soreness during post-exercise inflammation.
(Raimondo et al.,	Murine and human macrophages	Laboratory experiment	Limon extracellular vesicles	Citrus limon has anti-inflammatory

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Results of a review of the effects of limon on oxidative stress and inflammation

Author	Sample Characteristics	Study Design	Intervention	Results
2022)	obtained from healthy donors were isolated and purified.		isolated from citrus limon juice. The juice is sequentially centrifuged. Furthermore, 600 µg of vesicles were obtained from 250 mg of citrus limon juice.	properties both in vivo and ex vivo by inhibiting inflammation through NF-κB signaling.
(Bao et al., 2020)	75 rats were randomly divided into 5 groups: control group, swimming group, vitamin c group, low dose limon peel flavariod group, high dose limon peel flavariod group. Each group consisted of 15 rats.	Experimental	Subjects in the vitamin C group were given intragastric vitamin C solution at a dose of 100 mg/day. The limon peel flavanoid group was given a dose of 50 and 100 mg/day. All groups did 30 minutes of swimming practice during the first week then added 10 minutes the following week. The exercises were carried out three times/week for 4 weeks	The content of flavonoids in limon peel has anti-oxidant properties and has the potential to reduce MDA levels after exercise.
(Fang et al., 2019)	A total of 16 healthy male and female pigs aged 15 weeks with a body weight of 37-49 kg. The samples were divided into four groups, namely the low-fat diet + green tea group, the high-fat diet + green tea group, the low-fat diet + green tea + citrus limon group, and the high-fat diet + green tea + citrus limon group.	Experimental	The dose of green tea given is 190 mg/kg/day) and the dose of citrus limon is 190 mg/kg/day.	High-fat diet + green tea + citrus limon can reduce malondialdehyde (MDA) levels after 1 hour and 2 hours post-intervention.

## Discussions

The main aim of the research of this systematic review is to analyze and highlight the potential of citrus limon in reducing inflammation and free radicals after physical exercise. Citrus limon contains flavonoids which have antioxidant properties (Mahmoud et al., 2019). The chemical structure of citrus limon with the chemical formula C<sub>6</sub>H<sub>8</sub>O<sub>7</sub> can be seen in Figure 2. It is known that physical activity increases the production of Reactive Protein Species (ROS) (McKeegan et al., 2021). Excessive increase in ROS production can cause damage to muscle fibers which will lead to fatigue (Wang et al., 2021). On the other hand, the presence of small stimuli from a low increase in ROS production is able to express endogenous antioxidants (Eddaikra & Eddaikra, 2021; Simioni et al., 2018). However, if physical activity is carried out intensely, it will cause an imbalance between excessive ROS production and the oxidant defense system, which is called oxidative stress (Hajam et al., 2022). Oxidative stress can be identified by examining biomarkers such as examining levels of Malondialdehyde (MDA) in the blood (Cherian et al., 2019).

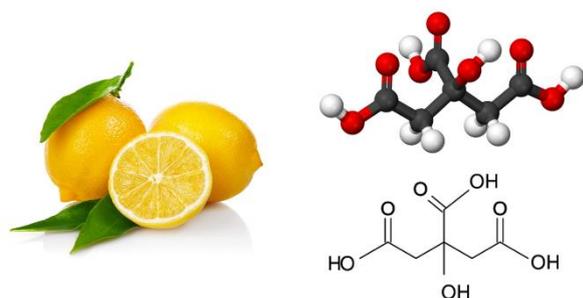


Figure 2. Chemical Structure of Citrus Limon

The idea that citrus limon contains antioxidant properties is supported by a study conducted on male mice reporting that giving citrus limon essential oil intervention at a dose of 50 mg/kg after swimming practice sessions in five sessions/week for four weeks was able to reduce triglycerides and MDA levels (Norouzi et al., 2020). The results of this study were reinforced by a study conducted on rats which reported that giving citrus limon peel flavonoids at doses of 50 and 100 mg/day after swimming practice, 3 times/week, for 4 weeks had the potential to reduce MDA levels (Bao et al., 2020). Furthermore, research (Fang et al., 2019) reported that a high-fat diet added to green tea and citrus limon at a dose of 190 mg/kg/day given to pigs was able to reduce MDA levels. The antioxidant properties of citrus limon are related to its chemical structure, especially the presence of a hydroxyl group (OH) which plays a role in protecting free radical injury through a radical scavenging mechanism (Simioni et al., 2018). Physiologically, another interesting thing is that the flavonoids contained in citrus limon work by increasing mitochondrial calcium (Ca<sup>2+</sup>) ions in cells, causing cell membrane hyperpolarization. So that an increase in mitochondrial Ca<sup>2+</sup> has the potential to reduce oxidative stress (Overdevest et al., 2018).

Furthermore, one of the main sources of oxidative stress is the immune system, and inflammation is the main reaction of the immune system to return cells damaged by intense exercise back to normal (Simioni et al., 2018). Indeed, when the cells of an organ are damaged, the immune system becomes active (Marshall et al., 2018). The cells will stimulate the macrophages to increase the production of pro-inflammatory and anti-inflammatory cytokines (Zhang & An, 2007). It has been reported that TNF-α is a part of the pro-inflammatory cytokine that triggers muscle pain (Ayubi, Purwanto Bambang, et al., 2022; Fernández-Lázaro et al., 2020; Nanavati et al., 2022). In this regard, citrus limon which has anti-inflammatory properties can be

an intervention strategy in controlling uncontrolled inflammatory processes due to intense exercise. A study reported that supplementation of citrus limon extract at a dose of 400 mg/day before and after exercise can prevent and reduce pain intensity, and prevent a decrease in muscle strength due to muscle damage and post-exercise inflammatory processes (Buchwald-Werner et al., 2018). The results of this study were reinforced by a study that reported that administering massage therapy interventions using topical oil with a citrus limon base after exercise was able to reduce muscle pain due to the inflammatory process (Harahap et al., 2023). Furthermore, a laboratory study recently reported that citrus limon extracted via hydrodistillation using a clevenger type tool has anti-inflammatory properties that have the potential to reduce inflammation (Yang et al., 2023). This study was also reinforced by a laboratory study which reported that citrus limon juice obtained from the results of isolated and centrifuged limon extracellular vesicles has anti-inflammatory properties both in vivo and ex vivo by inhibiting inflammation through NF-kB signals (Raimondo et al., 2022). Regarding NF-kB signaling, NF-kB is initially active when tissue damage occurs and then plays an important role in mediating inflammation, especially the secretion of pro-inflammatory cytokines such as TNF-a (Acar et al., 2018). So, if NF-kB signaling is inhibited using citrus limon, it will also affect TNF-a secretion and reduce muscle soreness.

So, the content of flavonoids contained in citrus limon which have anti-oxidant properties can reduce oxidative stress and anti-inflammatory properties in citrus limon can reduce uncontrolled inflammation due to intense exercise. Furthermore, for more details regarding the benefits of citrus limon in reducing oxidative stress and inflammation, see Figure 3.

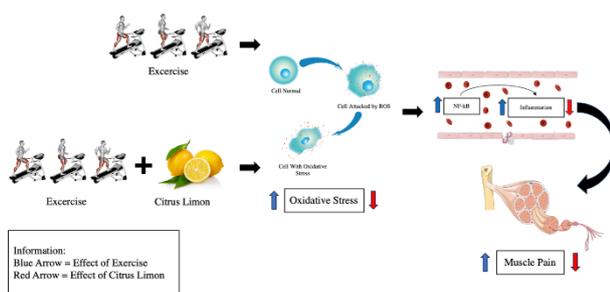


Figure 3. Mechanism of Action of Citrus Limon to Reduce Oxidative Stress and Inflammation

## Conclusions

The flavonoid content found in citrus limon has anti-oxidant properties that can reduce oxidative stress. Furthermore, the anti-inflammatory properties of citrus limon can reduce uncontrolled inflammation due to physical activity and intense exercise. In this case, citrus limon works by inhibiting inflammation through NF-kB signaling and reducing inflammation by suppressing the secretion of pro-

inflammatory cytokines such as TNF-a. Reducing inflammation can potentially reduce the intensity of muscle pain. We recommend that citrus limon be used in individuals to reduce oxidative stress and inflammation caused by physical activity and intense exercise.

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## Conflicts of Interest

The authors declare no conflict of interest

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