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Antioxidant properties of lemon essential oils: a meta-analysis of plant parts, extraction methods, dominant compounds, and antioxidant assay categories

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Abstract

Recent studies have explored the antioxidant properties of lemon essential oil (LEO), taking considering factors like plant part, extraction methods, and antioxidant assay. However, due to varied results and limited precision in individual studies, our meta-analysis aims to offer a comprehensive understanding across different experiments, irrespective of location or time. Out of 109 scientific articles published between 1947 and 2024, only 28 successfully validated their data on differences in antioxidant capacity and IC_{50} , using weighted averages of Hedges' d in meta-analysis. A meta-analysis revealed several key findings: (i) lemon leaf and peel extracts have higher IC_{50} compared to controls, whereas whole plant extracts show lower values ($p < 0.001$); (ii) the maceration preserves antioxidant properties better than hydro-distillation and Soxhlet extraction ($p < 0.001$); (iii) LEO require higher concentrations to achieve comparable free radical inhibition as the standard controls such as AsA, BHT, and quercetin, suggesting lower antioxidant efficiency. This was supported by IC_{50} result, which showed no significant difference between LEO and other compounds like thymol, *Thymus vulgaris* EO, and *Citrus aurantium* EO. However, compared to AsA, BHT, limonene, and trolox, the inhibition efficacy was significantly lower ($p < 0.01$). These findings consistently demonstrated significant antioxidant activity across multiple assays, including ABTS, β -carotene bleaching, DPPH, and FRAP ($p < 0.01$). Notably, the predominant components of LEO including α -linoleic acid, D-limonene, limonene, L-limonene, neryl acetate, sabinene, and Z-citral, which demonstrate significant potency as antioxidant agent ($p < 0.01$). Specifically, limonene and Z-citral make substantial contributions to its antioxidant capacity ($p < 0.01$). Despite variations in purity among LEO extractions, there is potential for future enhancement through nanoemulsion. In conclusion, LEO show promise as an alternative antioxidant, with emphasis to selecting samples based on leaves or peels and employing maceration extractions for various antioxidant assays. Active components rich in terpenoids, such as limonene and Z-citral, are particularly noteworthy.

Highlights

1. Lemon leaves and peel are characterized by their highest observed antioxidant activity.
2. Maceration serves to maintain the antioxidant effectiveness of LEO.
3. Limonene (D/L) and Z-citral are noted for their exceptional antioxidant properties.

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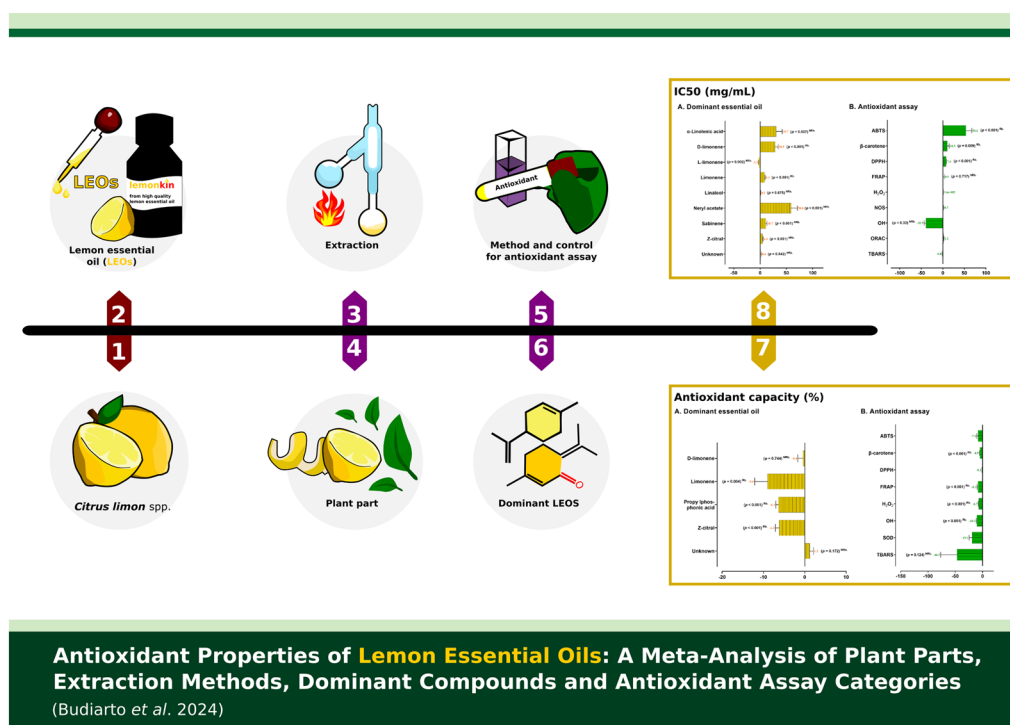
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Keywords BHT, Free radical, Limonene, Nanoemulsion, Z-citral

Graphical Abstract



Introduction

Various studies, including mechanistic, observational, and intervention approaches, consistently indicate that increasing the consumption of fruits, vegetables, and whole grains is associated with a reduced risk of metabolic syndrome-related diseases, including neurodegenerative disorders, cardiovascular disease, type 2 diabetes, and cancer [1]. These diseases are primarily linked to systemic and low-grade chronic inflammation induced by oxidative stress. The bioactive compounds within fruits, vegetables, and whole grains play a pivotal role in preventing cellular oxidative damage by detoxifying free radicals, thereby reducing the incidence of such diseases [2, 3].

The lemon (*Citrus limon* or *Citrus limonium*) is a fruit tree belonging to the Rutaceae family [4, 5]. Global lemon production is projected to exceed 1.2 million tons in the next five years [6, 7]. Lemons are primarily cultivated in warm and temperate climates due to their sensitivity to low temperatures. The average proportion of lemon production for the world market from 2010 to 2020 are as follows: Argentina (25.4%), Spain (17.9%), the USA

(14.4%), Turkey (13.7%), Italy (8.69%), South Africa (6.11%), and other countries (15.3%) [8, 9]. Although lemon essential oil (LEO) accounts for only ~0.1% of the fresh lemon fruit biomass [8], the demand for LEO is very high, comprising approximately two-third of the total world essential oil (EO) demand, which includes data from oranges and mint, especially for use in food and aromatherapy [10]. LEO consists of terpene/terpenoid-based compounds, aldehydes, ketones, esters, and phenols [11–16]. D-limonene, a terpene found in LEO, provides its characteristic lemon scent [17, 18]. Additionally, LOE contains compounds such as α/β -pinene, sabinene, p-cymene, γ -terpinene, α -terpineol, neral, geranial, β -bisabolene, neryl acetate, geranyl acetate, and other unidentified compounds [11, 19, 20]. Moreover, lemons are rich in ascorbic acid (vitamin C), tocopherols, tocotrienols (which belong to the vitamin E group), and minerals (Se, Zn, Cu, Fe, and Mn) [21–24]. These components are integral parts of the human diet, and citrus fruits serve as excellent sources of antioxidants, which have the potential to help prevent cellular oxidative damage [13, 25]. However, the variability in

studies on antioxidants from LEO is attributed to differences in the composition of essential oils. Recent studies have indicated that limonene from the peel exhibits higher antioxidant activity compared to the control [26]. Conversely, other studies on LEO, particularly limonene, report lower antioxidant activity compared to the control [4]. These variations in IC_{50} values are also influenced by external factors affecting the treatment. Consequently, a detailed meta-analysis is necessary to thoroughly review the external factors impacting the effectiveness of LEO as antioxidants.

Current research explores antioxidant properties, which vary in EO due to differences in plant parts, extraction methods, dominant compounds in EO, antioxidant assays, comparisons with different controls, measured free radicals, and various factors that significantly influence the antioxidant capacity and IC_{50} [4, 21, 27–30]. For example, IC_{50} values using ABTS indicated inhibition at 28 mg/mL of LEO [12], whereas similar tests reported inhibition at 2.97 mg/mL for the same [25]. This difference is attributed to variation in lemon varieties and methods of EO extraction, resulting in differing EO compositions [12, 25]. Both studies notably employed steam-distillation to extract samples from fruit peels [12, 25]. Discrepancies also stem from diverse EO sources, with the antioxidant capacities of samples from fruits and seeds (steam-distillation and maceration, respectively) showing higher values compared to controls (quercetin and distilled water) [31, 32].

Factors such as plant parts, extraction methods, dominant compounds, and antioxidant assays of LEO are crucial. Variations in data arise due to combinations of these four factors, as seen in previous findings related to the variations in temperature and storage duration of kaffir lime EO [33, 34]. However, conventional articles are often lack sufficient scientific justification. Therefore, statistical methods like meta-analysis are necessary. Moreover, for a comprehensive understanding of LEO antioxidant properties. Meta-analysis integrates previous studies focusing on lemon with interventions involving antioxidant assays of LEO, comparisons usually involving control-treatment, and outcomes represented by antioxidant values. It also considers various research methods, including randomized, comparative (direct/indirect), and nested (clustered) designs [33].

As previously explained, LEO demonstrate notable antioxidant properties due to their effectiveness in combating free radicals and their potential roles in addressing cancer, inflammation, and diabetes [35]. Despite the growing interest in natural antioxidants for their health benefits, there remains a need for a comprehensive understanding of the antioxidant properties specific to lemon essential oils. This study presents a structured

approach to integrating previous research findings, aiming to enhance our understanding of LEO antioxidant properties, attributed to its diverse range of bioactive compounds. This analytical method has the potential to offer valuable insights that can guide future research efforts and explore potential applications in food and therapy.

Materials and methods

Studies search and screening

The PRISMA-P checklist was utilized as a guideline for conducting the meta-analysis of in vitro antioxidant activity on LEO [36–38]. A systematic literature search was performed on reputable search engines, including Google Scholar, NCBI, ProQuest, Scopus, and ScienceDirect, to identify trustworthy studies published between 1947 and 2024. Search terms were structured according to the previous PICO framework, utilizing keywords such as (“*Citrus limon*” OR “*Citrus limonum*” OR “lemon”) AND (“essential oil” OR “EO” OR “limonene”) AND (antioxidant* OR “in vitro” AND antioxidant) [39, 40]. One researcher conducted the searches independently, reviewed titles, and screened for duplicate articles within the databases. Subsequently, two other independent researchers evaluated the title and abstract content related to the main topic for further selection.

Evaluation and selection of studies

The remaining articles that passed the initial rough selection stage were then subjected to an experimental-based research article selection process, utilizing information from the full-text articles. As a result, other articles such as books, book chapters, review articles, systematic reviews, non-peer-reviewed articles (including proceedings), and gray literature were excluded from consideration. A total of 109 scientific articles were selected for full-text evaluation. Subsequently, these articles were organized using the Mendeley Desktop (ver. 1.19.8) reference manager, and their assessment strictly adhered to the inclusion criteria outlined below: (a) original research studies indexed in reputable publishers, with a digital object identifier (DOI) or a globally accessible uniform resource locator (URL), (c) providing information about lemon as an essential oils and its impact on in vitro antioxidant activity, encompassing both antioxidant capacity or IC_{50} , and (c) the chosen articles were required to present quantitative data, in tabular and graphical formats. Non-experimental studies, such as surveys and modeling or estimation studies (not directly measured), were also excluded. Furthermore, a supplementary search was conducted through the cited references of the selected studies to ensure the initial search did not overlook relevant studies. The selection, extraction, validation, and

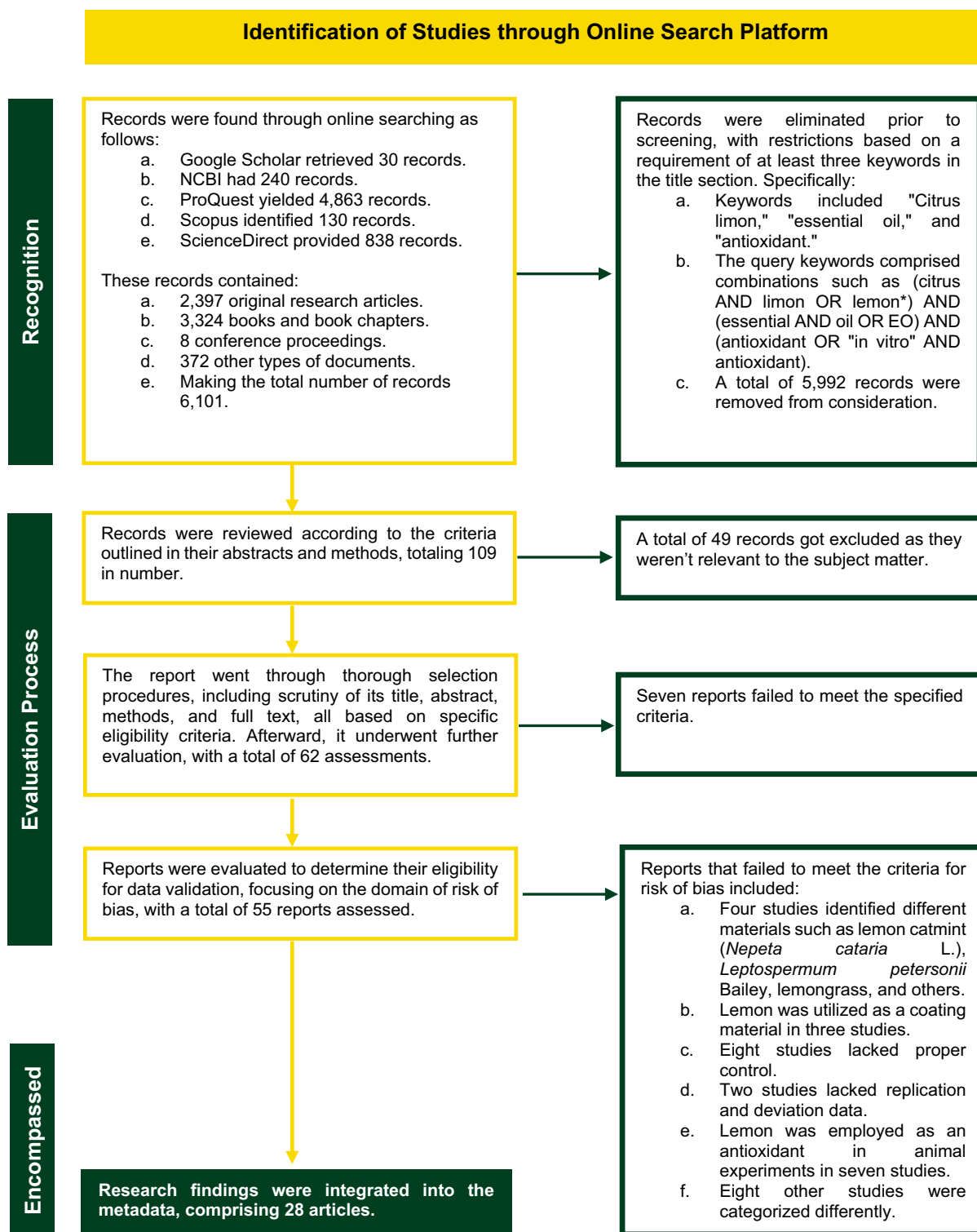


Fig. 1 Identification, screening, weighting (eligible full text of study), and selection processes of various primary research sources relevant to the topic of antioxidant properties of lemon essential oils [45]

standardization processes involved five investigators. Any disagreements were resolved through discussion. Details of the selection process are depicted in Fig. 1. A total of 55 articles were obtained that met the selection criteria.

Data tabulation and validation

Details such as lemon variants, parts of the lemon plant, extraction methods, dominant essential oils components, control or comparator compounds, antioxidant assay methods, compounds measured in the antioxidant test, and study sources were included. Data from 55 articles were tabulated in Microsoft Excel; however, only 28 articles were successfully inputted and validated. The remaining articles consisted of 4 studies that reported the use of different EO, 3 studies related to the use of LEO as packaging material, 8 studies that did not mention controls, 2 studies that did not report replications and standard deviations, 7 studies reporting the use of lemon as an antioxidant in experimental animals (in vivo), and finally, 8 studies that were discarded for other reasons. Information related to the 28 studies used in the meta-analysis is tabulated in Table 1. Verified data were categorized based on the differences in antioxidant studies, namely antioxidant capacity and inhibitory concentration (IC_{50}) groups. Antioxidant capacity (in %) refers to the compounds ability to protect cells from damage by free radicals and oxidative stress, while IC_{50} represents the concentration of a compound capable of reducing oxidation activity by 50% (in mg/mL) [41–44]. Higher antioxidant capacity value indicate greater effectiveness, whereas lower IC_{50} values are more effective [42]. All antioxidant capacity values were transformed into percentages (%) and IC_{50} values were standardized in mg/mL. The entirety of the selection process was conducted to ensure only credible study was chosen, which was subsequently presented in Fig. 1.

Data information details

Lemon varieties included *C. limon* (unspecified varieties), *C. limon* L. Burm. F., *C. limon* L. Burm., *C. limon* cv. Eureka, *C. limon* cv. Lisbon, *C. limon* cv. Pompia, *C. limon* cv. Rasraj, and *C. limonum* L. The lemon plant parts used for LEO extraction included fruit, leaf, peel, whole plant, residual biomass (comprising leaves, twigs, and small branches), and seed. Various extraction methods were utilized, including cold pressing, hydroalcoholic, hydro-distillation (HD), maceration, microwave-assisted hydro-distillation (Ma-HD), solvent-free microwave extraction (SFME), Soxhlet extraction (SD), steam-distillation (SEt), and supercritical fluid extraction.

Controls used in the study included ascorbic acid (AsA), butylated hydroxytoluene (BHT), *Citrus aurantium* essential oil (CaEO), limonene, quercetin, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic

acid (trolox or vitamin E analog), *Thymus vulgaris* essential oil (TvEO) and distilled water. Antioxidant assays were categorized into assessing the 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), measuring of 1,1-diphenyl-2-picrylhydrazyl (DPPH), β -carotene bleaching, ferric reducing antioxidant power (FRAP), hydrogen peroxide measurement (H_2O_2), nitric oxide scavenging activity (NOS), hydroxyl radical measurement (OH), oxygen radical absorbance capacity (ORAC), superoxide dismutase (SOD), and thiobarbituric acid reactive substances (TBARS).

Compounds measured as reference for antioxidant reactions included ABTS radical cation ($ABTS^{+\bullet}$), DPPH free radical ($DPPH^{\bullet}$), ferric ion transformation (FeT), superoxide anion radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), peroxy radical ($ROO^{\bullet}$), hydroxyl radical ($^{\bullet}HO$), lipid peroxidation (LPO), and nitric oxide radical ($^{\bullet}NO$).

Publication bias

Risk of bias (ROB) from the studies used in the meta-analysis was evaluated using the Cochrane Collaboration assessment method [46]. A total of 28 chosen studies were analyzed: 8 papers reported on antioxidant capacity and 19 papers reported on IC_{50} , along with 1 paper reporting on both aspects (Fig. 2). This bias assessment was based on criteria such as bias from randomization of LEO vs. control (D1), bias due to deviations from intended interventions (D2), bias due to missing data (D3), bias due to measurement of outcomes (D4), and bias due to selection of the reported results (D5). Each criterion was assessed step-by-step for each study, with a score of 3 indicating “low risk”, a score of 2 indicating “some concerns”, and a score of 1 indicating “high risk”. These scores were then used to determine an overall risk of bias for each study. The individual assessments for each criterion were summarized in a table and inputted into the Robvis (Risk-of-bias VISualization) website application to generate traffic light plots (as individual plots of the studies) and weighted bar plots (as summary ROB) [45, 47]. The overall risk of bias is depicted in Fig. 2.

Meta-analysis model and validation

The meta-analysis began by defining the control groups, which including positive controls such as synthetic antioxidants like BHT, as well as other natural antioxidants like essential oils from *T. vulgaris* and others. Meanwhile, the control group comprised lemon essential oils obtained through extraction methods. The basic data utilized for the analysis included replicates or the number of samples for each treatment, the averages or mean values from in vitro antioxidant, and the standard deviations. Subsequently, the comparison calculation between the control and treatment groups using LEO was conducted

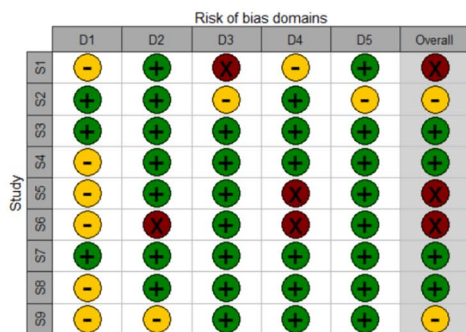
Table 1 Detailed data and information on plant part, extraction method, control, antioxidant assay, and measured compounds in LEO for in vitro antioxidant activity

Lemon	Plant part	Extraction method	LEO (%)	Control	Antioxidant assay	Measured compound	Study
Antioxidant capacity (%)							
<i>C. limonum</i> L.	Fruit	Maceration	–	Water	DPPH	DPPH [•]	S1 [31]
<i>C. limon</i>	Peel	SD	D-limonene (74)	–	DPPH	DPPH [•]	S2 [55]
<i>C. limon</i>	Leaf	SEt	Z-Citral (53.2)	AsA, Aspirin, and BHT	DPPH, OH, and SOD	DPPH [•] , [•] HO, and O ₂ ^{•−}	S3 [14]
<i>C. limon</i> L. Burm	–	–	D-limonene (70)	Trolox	DPPH	DPPH [•]	S4 [4]
<i>C. limon</i>	Peel	SD	Limonene (61.4)	AsA	DPPH	DPPH [•]	S5 [26]
<i>C. limon</i>	Peel	SD	Limonene (72.4)	CaEO	ABTS and DPPH	ABTS ^{•+} and DPPH [•]	S6 [56]
<i>C. limon</i>	Fruit, Peel, and Seed	Maceration	–	Quercetin	DPPH	DPPH [•]	S7 [32]
<i>C. limon</i>	Leaf	HD and SEt	Limonene (60.5–73.6)	BHT	DPPH and TBARS	DPPH [•] and LPO	S8 [57]
<i>C. limon</i>	Whole plant	HD	–	BHT	β-carotene bleaching, DPPH, OH, and H ₂ O ₂	ROO [•] , DPPH [•] , [•] HO, and H ₂ O ₂	S9 [58]
IC ₅₀ (mg/mL)							
<i>C. limon</i>	Peel	–	–	Limonene	ABTS, FRAP, NOS, OH, and ORAC	ABTS ^{•+} , FeT, [•] NO, [•] HO, and ROO [•]	T1 [12]
<i>C. limon</i>	Leaf	SD	Limonene (33.6)	AsA	DPPH	DPPH [•]	T2 [59]
<i>C. limon</i>	Peel	HD	Limonene (55)	BHT	DPPH	DPPH [•]	T3 [60]
<i>C. limon</i> cv. Rasraj	Whole plant	HD	L-limonene (60.3)	–	DPPH, FRAP, OH, and TBARS	DPPH [•] , FeT, [•] HO, and LPO	T4 [13]
<i>C. limon</i>	Leaf	SEt	Z-Citral (53.2)	AsA and BHT	DPPH	DPPH [•]	T5 [14]
<i>C. limon</i>	Leaf	HD	–	BHT	H ₂ O ₂	H ₂ O ₂	T6 [61]
<i>C. limon</i> L. Burm	Peel	SD	D-limonene (70.7)	Limonene	ABTS, β-carotene, and DPPH	ABTS ^{•+} , ROO [•] , and DPPH [•]	T7 [25]
<i>C. limon</i>	Peel	HD	D-limonene (48.6–53.4)	AsA	DPPH and FRAP	DPPH [•] and FeT	T8 [62]
<i>C. limon</i> cv. Pompia	Peel	SD	Limonene (29.8)	Trolox	ABTS and DPPH	ABTS ^{•+} and DPPH [•]	T9 [63]
<i>C. limon</i> cv. Lisbon	Peel	HD, Ma-HD, and SFME	Limonene (58.6–63.2)	AsA and BHT	DPPH	DPPH [•]	T10 [64]
<i>C. limon</i>	Leaf	SD	D-limonene (52.7) and Sabinene (36.1)	Thymol and TvEO	β-carotene bleaching and DPPH	[•] HO and DPPH [•]	T11 [65]
<i>C. limon</i> cv. Eureka	Peel	Cold pressing	Limonene (67.1)	–	DPPH and FRAP	DPPH [•] and FeT	T12 [19]
<i>C. limon</i>	Leaf	HD	Linalool (30.6)	AsA and BHT	DPPH	DPPH [•]	T13 [28]
<i>C. limon</i>	Residual biomass	Hydroalcoholic and HD	α-Linolenic acid (20.8)	CaEO	DPPH	DPPH [•]	T14 [66]
<i>C. limon</i>	Whole plant	HD	Limonene (30) and Neryl acetate (29.5)	AsA	ABTS and DPPH	ABTS ^{•+} and DPPH [•]	T15 [67]
<i>C. limon</i>	Leaf	HD	Limonene (33.9)	Trolox	DPPH	DPPH [•]	T16 [11]
<i>C. limon</i> cv. Pompia	Peel	SD	Limonene (82.1)	Trolox	β-carotene bleaching	ROO [•]	T17 [68]
<i>C. limon</i> cv. Pompia	Seed	Supercritical fluid	–	Trolox	ABTS	ABTS ^{•+}	T18 [5]
<i>C. limon</i> L. Burm. F	Peel	HD and SEt	Limonene (28.3–76.8)	BHT and Trolox	DPPH	DPPH [•]	T19 [21]
<i>C. limon</i>	–	–	Limonene (64.5)	AsA and Trolox	DPPH	DPPH [•]	T20 [27]

AsA ascorbic acid, BHT butylated hydroxytoluene, CaEO *C. aurantium* essential oil, FeT Ferric ion transformation, O₂^{•−} superoxide radical, H₂O₂ hydrogen peroxide, [•]HO hydroxyl radical, HD hydro-distillation, LEO predominant compound in lemon essential oil, LPO level of lipid peroxidation, Ma-HD microwave-assisted hydro-distillation, [•]NO nitric oxide radical, ROO[•] peroxy radical, SFME solvent-free microwave extraction, SD steam-distillation, SEt Soxhlet extraction, Trolox 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, TvEO *T. vulgaris* essential oil

A. Risk of bias arising from individual studies

A.1. Antioxidant capacity



Domains:

- D1: Bias due to randomisation.
- D2: Bias due to deviations from intended intervention.
- D3: Bias due to missing data.
- D4: Bias due to outcome measurement.
- D5: Bias due to selection of reported result.

Judgement

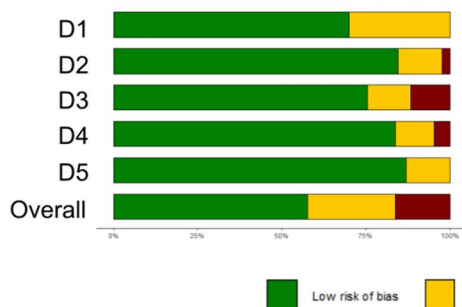
- High
- Some concerns
- Low

A.2. IC50



B. Summary of risk of bias

B.1. Antioxidant capacity



B.2. IC50

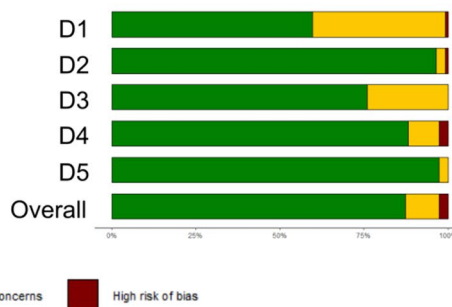


Fig. 2 Individual and summary of ROB from selected scientific articles in meta-analysis data

employing effect size comparison through Hedges' d (d) [48]. The variables for meta-analysis were calculated according to the following equation:

$$d = \frac{\bar{x}_{LEOs} - \bar{x}_c}{S_p} \times \left(1 - \frac{3}{4(N_{LEOs} + N_c - 2) - 1} \right) \quad (1)$$

The formula for the Hedges' d coefficient (Eq. 1) where $\bar{x}_c$ is the mean value of antioxidant activity from the control, $\bar{x}_{LEOs}$ is the mean value of antioxidant activity from the LEO, and S_p is the combined standard deviation of the control and experimental groups. Meanwhile, the formula for the variance of Hedges' d ($var(d)$) is delineated as Eq. 2.

$$\text{var}(d) = \frac{n_{LEOs} + n_c}{n_{LEOs} \times n_c} + \frac{d^2}{2(n_{LEOs} + n_c)} \quad (2)$$

The explanation stated that if n_c represented the sample size of the control group and n_{LEOs} represented the sample size of the treatment group (LEO). Subsequently, the calculation of the sum of mean difference (SMD) was formulated as Eq. 3.

$$\text{SMD} = \sum_{i=1}^n d_i \quad (3)$$

In the study, the notation d_i was used to represent the effect size of each individual study, while n denoted the total number of studies. If the outcome of the SMD was positive, it meant that the treatment surpassed the control, and conversely if it was negative. The absolute value of SMD served as a standard measure to assess the magnitude of the effect size retroactively: (SMD > 0.2) indicated a small effect, (SMD > 0.5) suggested a medium effect, and (SMD > 0.8) indicated a large effect [49, 50]. Finally, the confidence interval (CI) for the Hedges' d coefficient was determined using the formula provided in Eq. 4 [51, 52].

$$\text{CI} = d \pm Z \times \sqrt{\text{var}(d)} \quad (4)$$

In which d represented the Hedges' d coefficient, Z stood for the z-score for a 95% confidence level, and SE indicated the standard error of the Hedges' d coefficient.

If the confidence interval did not cover the null effect size, the effect size calculated was considered statistically significant [52, 53]. A fail-safe number (FsN) was determined to identify publication bias resulting from excluded insignificant studies. Evidence of publication bias was suggested if FsN exceeded [5 times the number of studies included] + 10. Another calculation was performed to determine the cumulative effect size for various variables categories, such as lemon, based on different genotype varieties and samples of LEO obtained from different plant parts, extraction methods, antioxidant assays, variations in the control group, and marker compounds used for antioxidant measurement. All calculations involving Hedges' d coefficient were executed using OpenMEE for conducting a user-friendly meta-analysis in ecology and evolutionary biology [54].

Results

The antioxidant capacity of LEO is significantly lower than the control, with a large sum of effect size, $\text{SMD} = -3.22 \pm 2.07$ ($p < 0.001$, Table 2). LEO sourced from both the fruit and seed parts exhibit significantly higher antioxidant capacities ($p < 0.01$), whereas the leaf and whole plant parts show lower antioxidant capacities ($p < 0.01$). All four parts have robust FsN values

($\text{FsN} \geq \text{FsC}$). However, parts like peel and unknown portions are not significant ($p > 0.05$), indicating a non-robust model ($\text{FsN} < \text{FsC}$).

Furthermore, extraction methods such as hydro-distillation, maceration, Soxhlet extraction, and steam-distillation show significant values and large SMDs ($p < 0.01$ and $\text{SMD} > 0.8$), with only steam-distillation showing non-robustness. Specifically, maceration exhibits a positive SMD. Subgroup analysis conducted on groups of predominant LEO compositions (refer to Fig. 3A) reveals that limonene and Z-citral exhibit significantly lower antioxidant capacities with substantial SMDs in a robust model ($p < 0.01$ and $\text{SMD} > 0.8$) compared to the control group.

Another categorization of meta-analysis compares LEO with AsA, aspirin, BHT, quercetin, and distilled water, all showing significant differences with high SMDs ($p < 0.01$ and $\text{SMD} > 0.8$), although positive signs are observed only in the comparison with quercetin or distilled water. A non-robust model is observed in the comparison with aspirin ($\text{FsN} < \text{FsC}$). Subgroup analysis of variations in antioxidant assay for antioxidant capacity (Fig. 3B) shows that β -carotene bleaching, FRAP, H_2O_2 , and OH exhibit significance and high SMDs ($p < 0.01$ and $\text{SMD} > 0.8$), despite showing negative values compared to the control. Similarly, subgroups measured compound H_2O_2 , $\text{HO}^\bullet$, and $\text{ROO}^\bullet$, as well as ferric ion transformation measurements, are significant with large SMDs ($p < 0.001$ and $\text{SMD} > 0.8$). In all these compound groups, negative values indicate that antioxidant activity of LEO is lower compared to the control.

Measurement of antioxidant IC_{50} as recorded in Table 3 shows that LEO have a higher IC_{50} compared to the control, at $\text{SMD} = 5.99 \pm 3$ ($p < 0.01$). Subsequent subgroup analysis of plant parts indicates that leaf, peel, residual biomass, and the unknown group exhibit significantly high SMD compared to the control ($p < 0.01$ and $\text{SMD} > 0.8$). Only residual biomass does not show not robustness ($\text{FsN} < \text{FsC}$).

Extraction methods for LEO, such as hydro-distillation, microwave-assisted hydro-distillation, solvent-free microwave extraction, and steam-distillation, show significant and large SMD compared to the control ($p < 0.05$ and $\text{SMD} > 0.8$). Microwave-assisted hydro-distillation and solvent-free microwave extraction yield non-robust models ($\text{FsN} < \text{FsC}$).

In Fig. 4A, the subgroup of analysis of LEO dominant compound indicated that α -linoleic acid, D-limonene, limonene, neryl acetate, sabinene, and z-citral are significant and have a higher SMD than the control ($p < 0.01$ and $\text{SMD} > 0.8$), except for L-limonene ($p = 0.002$ and $\text{SMD} = -2.2 \pm 2.79$). Robust models are observed only for D-limonene and limonene ($\text{FsN} > \text{FsC}$).

Table 2 Antioxidant capacity (%) of lemon essential oils based on plant part, extraction method, control group, and measured compound

	Ns	Study effect SMD ± ΔS	SEM	P value	Heterogeneity		FsN
					I ²	P value	
Antioxidant capacity (%)	89	− 3.22 ± 2.07 ^{Rb.}	0.529	< 0.001	87.6	< 0.001	5966
Plant part							
Fruit	11	5.41 ± 3.49 ^{Rb.}	0.89	< 0.001	62.7	0.003	383
Leaf	34	− 7.02 ± 2.97 ^{Rb.}	0.757	< 0.001	75.7	< 0.001	4029
Peel	14	0.9 ± 3.16 ^{NRb.}	0.807	0.264	83.6	< 0.001	10
Seed	5	4.99 ± 6.62 ^{Rb.}	1.69	0.003	80.2	< 0.001	60
Unknown	1	− 22.6 ± 25.8 ^{NRb.}	6.59	–	–	–	4
Whole plant	24	− 6.34 ± 2.63 ^{Rb.}	0.671	< 0.001	63.5	< 0.001	1974
Extraction method							
Hydro-distillation	38	− 6.64 ± 2.25 ^{Rb.}	0.575	< 0.001	65.2	< 0.001	5098
Maceration	21	4.84 ± 2.61 ^{Rb.}	0.666	< 0.001	71.2	< 0.001	1251
Soxhlet extraction	20	− 6.76 ± 3.99 ^{Rb.}	1.02	< 0.001	78.5	< 0.001	1332
Steam-distillation	9	− 0.64 ± 3.35 ^{NRb.}	0.854	0.454	80.4	< 0.001	0
Unknown	1	− 22.6 ± 25.82 ^{NRb.}	6.59	–	–	–	4
Control group							
Ascorbic acid	15	− 7.93 ± 5.34 ^{Rb.}	1.36	< 0.001	83.3	< 0.001	700
Aspirin	2	− 13 ± 18.4 ^{NRb.}	4.7	0.006	58.6	0.120	15
BHT	42	− 6.02 ± 2.17 ^{Rb.}	0.553	< 0.001	79.4	< 0.001	5917
<i>C. aurantium</i> EO	2	− 4.44 ± 12.01 ^{NRb.}	3.06	0.147	82.1	0.018	8
Distilled water	6	6.37 ± 4.45 ^{Rb.}	1.14	< 0.001	41.3	0.130	127
Trolox	1	− 22.6 ± 25.8 ^{NRb.}	6.59	–	–	–	4
Quercetin	15	4.13 ± 2.85 ^{Rb.}	0.728	< 0.001	71.4	0.000	568
Unknown	6	0.14 ± 4.34 ^{NRb.}	1.11	0.898	82.4	< 0.001	0
Measured compound							
ABTS ⁺	1	− 7.94 ± 9.54 ^{NRb.}	2.43	–	–	–	3
DPPH [•]	54	− 0.24 ± 2.33 ^{NRb.}	0.596	0.692	87.6	< 0.001	40
FeT	6	− 8.28 ± 5.75 ^{Rb.}	1.47	< 0.001	45.5	0.102	136
H ₂ O ₂	12	− 6.67 ± 3.58 ^{Rb.}	0.91	< 0.001	53.8	0.014	514
HO [•]	7	− 10.3 ± 4.63 ^{Rb.}	1.18	< 0.001	< 0.001	0.849	196
LPx	2	− 46.5 ± 119 ^{NRb.}	30.2	0.124	84.3	0.012	16
ROO [•]	6	− 4.85 ± 3.36 ^{Rb.}	0.858	< 0.001	35	0.174	112
O ₂ ^{•−}	1	− 19.1 ± 21.8 ^{NRb.}	5.56	–	–	–	4

^{Rb.} and ^{NRb.} The robustness of the fail-safe N (FsN) according to Rosenthal at a 5% error threshold is ensured when “FsN ≥ 5Ns + 10” holds true based on the data observed for effect size

ABTS⁺ ABTS radical cation, BHT butylated hydroxytoluene, ΔS upper–lower, DPPH[•] DPPH free radical, EO essential oil, FeT Ferric ion transformation, FsN fail-safe N, H₂O₂ hydrogen peroxide, HO[•] hydroxyl radical, LPx Level of lipid peroxidation, Ns number of observed studies, O₂^{•−} superoxide radical, ROO[•] peroxy radical, SFME solvent-free microwave extraction, Trolox 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid

The control subgroup in the meta-analysis results indicates that AsA, BHT, CaEO, thymol, TvEO, trolox, and unknown substance are significant in IC₅₀ testing ($p < 0.05$ and SMD > 0.8). Non-robust models include CaEO, thymol, TvEO, and unknown (FsN < FsC). Meanwhile, IC₅₀ testing methods such as ABTS, β-carotene bleaching, and DPPH show robust models significantly higher than the control, consistent with SMD results ($p < 0.01$ and SMD > 0.8, Fig. 4B). Consistent with the

measured compound subgroup, robust models are observed for ABTS⁺, DPPH[•], and ROO[•] radicals ($p < 0.01$ and SMD > 0.8) compared to the control.

Discussion

Antioxidant capacity of LOE

Most citrus fruits, such as lemons, contain natural compounds that serve various functions in the human body, including antioxidant properties, anti-inflammatory

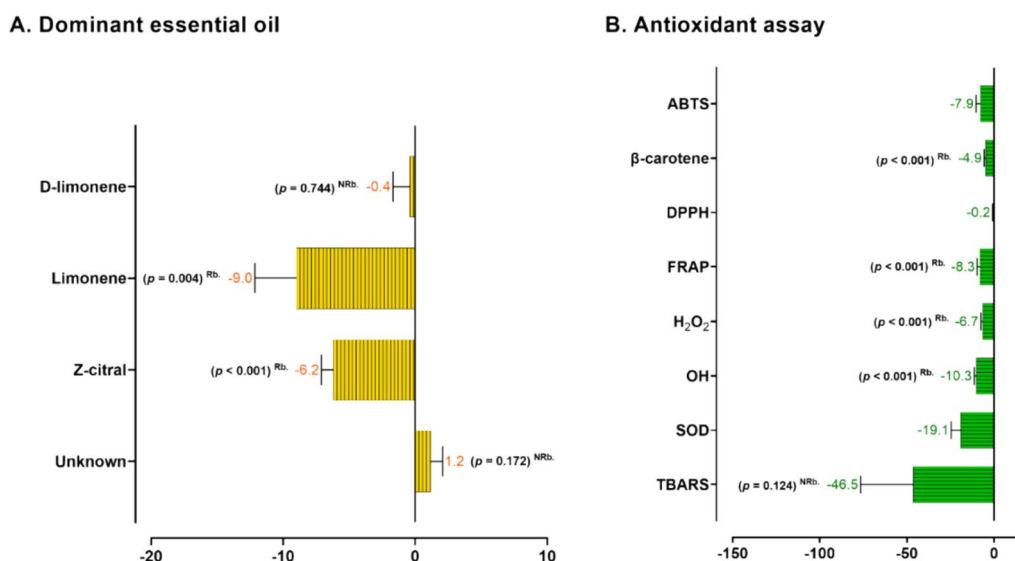


Fig. 3 Lemon antioxidant properties based on dominant essential oils (A) and type of antioxidant assay (B) on antioxidant capacity. Note that the fail-safe N calculation value is lower than Rosenthal test at alpha = 0.05 (robust = Rb.) and (not robust = NRb.) represents its opposite condition

effects, cancer prevention, and anti-aging benefits [32, 69]. Researchers in food science and medicine are increasingly interested in the protective effects of antioxidants against food damage and oxidative stress on the human body [70]. The antioxidant properties of lemon mainly come from AsA, essential oils, and phenols [24, 28]. In this meta-analysis, the antioxidant capacity of LEO was found to be lower compared to the positive control, which incidentally served as a benchmark. The positive controls used in this analysis mainly included AsA (n = 15), BHT (n = 42), and quercetin (n = 15), known for their strong antioxidant capacities both theoretically and practically (Table 2) [71, 72].

This discussion on antioxidant focuses on the ability of LEO within specific concentration ranges to inhibit free radical activity and similar measuring compounds. Higher percentage values indicate stronger antioxidant capacity, thus reflecting higher antioxidant activity of LEO. As indicated in Table 2, an antioxidant capacity of - 3.22 for LEO suggests significantly higher activity compare to the control ($|SMD| > 0.8$). However, it is essential to consider factors influencing this value, particularly since it may seem logical for control comparisons (such as AsA and BHT) to exhibit higher antioxidant capacities than LEO, despite LEO having lower to moderate purity levels (< 90%) [71, 73, 74].

IC₅₀ of LEO

Oxygen plays a central role in cellular metabolic processes, but it can gradually transforms into reactive compounds known as free radicals, leading to oxidative

reactions and eventual cell damage over time [75, 76]. Antioxidants counteract this process by neutralizing free radicals, thereby protecting biological functions [75]. This study explores how the varied antioxidant capacities found in LEO can effectively mitigate oxidative damage. A lower IC₅₀ value signifies greater efficiency in counteracting free radicals [42]. In comparison to the control group, the inclusion of LEO shows a significantly reduced impact on antioxidant capabilities, indicated by an SMD of 5.99.

Recent findings suggest that the antioxidant of LEO may be attributed to variations in their natural components [59]. Several research studies have suggested a correlation between IC₅₀ values and the composition of LEO [33]. This correlation aligns with the observed patterns in the results of the meta-analysis [32, 59, 77]. Active plant components with antioxidant properties, such as limonene, 3-carene, terpinolene, and other phenolic compounds found in LEO, were tested using the IC₅₀ of DPPH assay [25, 59, 62]. Factors such as dominant compounds and LEO significantly influence the activity of inhibiting 50% of free radicals. Therefore, further investigation into factors such as the plant part, extraction method, and antioxidant assay are crucial to comprehensive understanding.

Plant parts related to LEO antioxidant activity

The antioxidant capacities derived from fruit, leaf, plant, and seed of LEO exhibit a significant effect compared to the control, showing a large sum of effect sizes. When considering the direction of significance,

Table 3 IC₅₀ values (mg/mL) of antioxidant activity in lemon essential oil: an overview of plant part, extraction method, control group, and measured compound

	Ns	Study effect			Heterogeneity		FsN
		SMD ± ΔS	SEM	P value	I ²	P value	
IC ₅₀ (mg/mL)	64	5.99 ± 3 ^{Rb.}	0.765	< 0.001	88.5	< 0.001	7982
Plant part							
Leaf	11	5.04 ± 4.57 ^{Rb.}	1.17	< 0.001	88.5	< 0.001	243
Peel	43	6.74 ± 4.15 ^{Rb.}	1.06	< 0.001	61.8	0.106	3469
Residual biomass	2	30.7 ± 44.8 ^{NRb.}	11.4	0.007	83.8	< 0.001	16
Seed	1	144 ± 163 ^{NRb.}	41.6	–	–	–	4
Unknown	2	6.16 ± 4.2 ^{Rb.}	1.07	< 0.001	–	0.998	23
Whole plant	8	1.38 ± 8.65 ^{NRb.}	2.21	0.530	87.5	< 0.001	3
Extraction method							
Cold pressing	2	0.11 ± 2.27 ^{NRb.}	0.578	0.846	88.2	< 0.001	0
Hydroalcoholic	1	45.2 ± 51.3 ^{NRb.}	13.1	–	89.0	< 0.001	4
Hydro-distillation	30	6.71 ± 4.84 ^{Rb.}	1.24	< 0.001	90.8	< 0.001	1720
Ma-HD	2	30.9 ± 49.1 ^{NRb.}	12.5	0.014	66.9	0.082	16
SFME	2	86.5 ± 103 ^{NRb.}	26.3	0.001	46.6	0.171	16
Soxhlet extraction	8	1.93 ± 9.31 ^{NRb.}	2.38	0.416	–	0.845	7
Steam-distillation	14	11 ± 8.04 ^{Rb.}	2.05	< 0.001	–	–	611
Supercritical fluid	1	144 ± 163 ^{NRb.}	41.6	–	–	–	4
Unknown	7	2.78 ± 6.05 ^{NRb.}	1.54	0.072	87.2	< 0.001	33
Control group							
Ascorbic acid	17	27.5 ± 14.7 ^{Rb.}	3.75	< 0.001	90.1	< 0.001	1132
BHT	14	7.11 ± 5.89 ^{Rb.}	1.5	< 0.001	85.9	< 0.001	560
<i>C. aurantium</i> EO	2	30.7 ± 44.8 ^{NRb.}	11.4	0.007	61.8	0.106	16
Limonene	8	2.22 ± 6.11 ^{NRb.}	1.56	0.154	85.4	< 0.001	26
Thymol	2	12.7 ± 10.5 ^{NRb.}	2.67	< 0.001	–	0.543	15
<i>T. vulgaris</i> EO	2	10.7 ± 8.85 ^{NRb.}	2.26	< 0.001	–	0.749	15
Trolox	16	3.89 ± 6.59 ^{Rb.}	1.68	0.021	89.2	< 0.001	332
Unknown	6	– 1.37 ± 2.51 ^{NRb.}	0.642	0.032	60.5	0.027	19
Measured compound							
ABTS ⁺	8	53.6 ± 52.9 ^{Rb.}	13.5	< 0.001	91.4	< 0.001	216
DPPH [•]	43	7.41 ± 4.1 ^{Rb.}	1.05	< 0.001	88.4	< 0.001	4199
FeT	6	0.86 ± 9.32 ^{NRb.}	2.38	0.717	88.1	< 0.001	17
H ₂ O ₂	1	0.04 ± 3.2 ^{NRb.}	0.817	–	–	–	0
HO [•]	2	– 39.5 ± 160 ^{NRb.}	40.8	0.333	91.2	0.001	10
LPx	1	– 0.77 ± 3.32 ^{NRb.}	0.846	–	–	–	0
*NO	1	0.72 ± 3.3 ^{NRb.}	0.842	–	–	–	0
ROO [•]	5	6.51 ± 8.55 ^{Rb.}	2.18	0.003	84.2	< 0.001	64

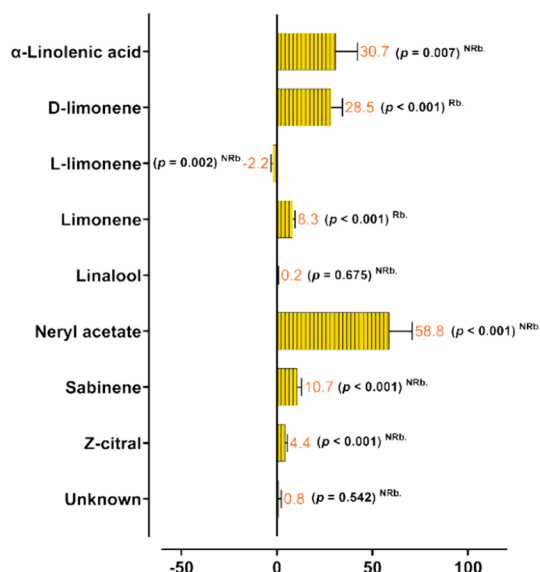
Rb. and NRb. The value of the fail-safe N (FsN) using Rosenthal at a 5% error level, where robust conditions are met if “FsN ≥ 5Ns + 10” from the observed data used in effect size

ABTS⁺ ABTS radical cation, BHT butylated hydroxytoluene, ΔS upper–lower, DPPH[•] DPPH free radical, EO essential oil, FeT Ferric ion transformation, FsN fail-safe N, H₂O₂ hydrogen peroxide, HO[•] hydroxyl radical, LPx Level of lipid peroxidation, Ma-HDs microwave-assisted hydro-distillations, *NO nitric oxide radical, Ns number of observed studies, O₂^{•–} superoxide radical, ROO[•] peroxy radical, SFME solvent-free microwave extraction, Trolox 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid

fruit and seed are higher than the control group, while leaf and whole plant are lower than the control. This is related to the type of control used in antioxidant capacity tests. Based on the meta-analysis data, where fruit and seed are compared to controls such as

distilled water, trolox, and quercetin. These three compounds are considered weak antioxidants compared to BHT [72, 78, 79]. Despite this, the findings confirm that the EO derived from different parts of the lemon exhibit better antioxidant capacities than other natural

A. Dominant essential oil



B. Antioxidant assay

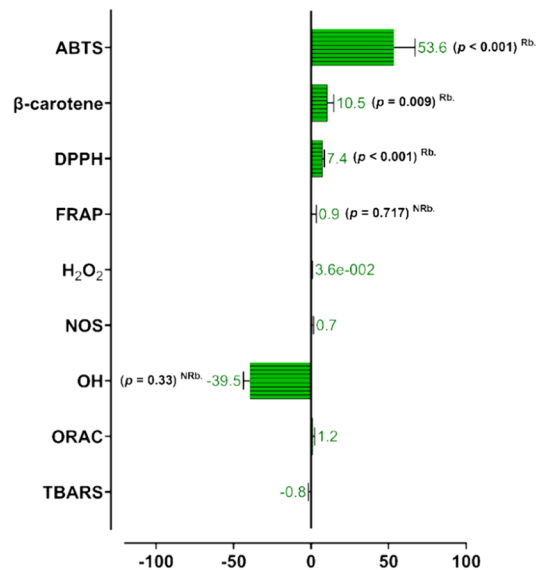


Fig. 4 Assessment of lemon antioxidant properties: influence of predominant essential oils (A) and type of antioxidant assay (B) on IC_{50} . Note that the robustness of the fail-safe N (FsN) calculation at $\alpha = 0.05$ differs from that of the Rosenthal value, with robust = Rb. ($FsN \geq 5Ns + 10$ known as FsC) and not robust = NRb. ($FsN < FsC$)

compounds, though still relatively low compared to BHT [21, 72].

As previously mentioned, the smallest IC_{50} value indicates the highest antioxidant potential in inhibiting of free radicals. Various LEO from parts of the lemon plant, including leaves, fruit peel, residual biomass, and unidentified parts, significantly exhibit a SMD in antioxidant properties. However, when compared to the control group, these values indicate that the minimum IC_{50} is much larger than that of the control, especially LEO from residual biomass. It is observed that leaf parts have the smallest IC_{50} compared to other parts. Most studies indicate that the main components of LEO derived from leaves are limonene and citral [11, 57]. Both compounds are reported to possess antioxidant activity [80–82]. Therefore, the plant part significantly determines the components of EO that can be extracted as antioxidant agents. Nearly all parts of the lemon can be utilized as a source of antioxidants, such as fruit, leaves, fruit peel, green biomass, and seeds. However, the extraction method also determines the quality and quantity of extracted EO.

Extraction methods related to LEO antioxidant activity

The antioxidant capacity of LEO, categorized by extraction method, follows this order: maceration > hydro-distillation > Soxhlet extraction. Methods like hydro-distillation and Soxhlet extraction, which involve

solvent heating for optimal exposure of LEO, typically employ temperatures ranging from 40 to 80 °C, or even up to 100 °C [29]. Research indicates that prolonged heat exposure and high temperature can diminish the antioxidant potency of EO [83–85]. Heat exposure can induce entropy change in heat-sensitive LEO, leading to deformation and degradation [83, 86, 87]. A meta-analysis suggests that employing heat in LEO extraction yields comparable outcomes, thus favoring maceration as a preferred option. However, maceration is less convenient and typically yields lower quantities compared to hydro-distillation and Soxhlet extraction [88, 89].

The effectiveness ranking of LEO as antioxidants, measured by IC_{50} , is as follows: SFME > Ma-HD > steam-distillation > hydro-distillation, in ascending order of strength compared to the control. This aligns with prior findings that high heat during extraction can induce damage and alter the chemical composition of LEO, thereby reducing their antioxidant efficacy [87]. Therefore, employing extraction methods that minimize heat exposure, such as cold pressing, may preserve the antioxidant effectiveness of LEO. However, this meta-analysis did not yield statistically significant results, indicated by the smallest SMD of 0.11 among the compared extraction methods [19].

Furthermore, in addition to focusing on the extraction method, the type of solvent used in the extraction process also significantly determines the components of LEO obtained. Although the meta-analysis did not present

data on the effect of solvent type on antioxidant properties due to limited information, recent research reports that lemon extract obtained through maceration using three different solvents resulted in the highest DPPH-IC₅₀ values in ethyl acetate > methanol > hexane, with sequential values of 0.09, 0.11, and 0.13 mg/mL [21]. This antiradical strength is attributed to the polarity of the solvents, with compounds extracted from polar solvents exhibiting relatively high antioxidant properties. Additionally, the freshness of the sample also affects the composition of LEO. For example, ether extract from fresh vs. dried *C. limon* peels resulted in a decrease in limonene from 76.8% to 28.3% [21]. Therefore, fresh sample preparation is preferable to obtain the primary component limonene, which is known to have diverse biological functions [17, 90].

The diversity of components in LEO is actually quite high due to the extraction process and sample freshness. However, a quick glance at the results of this meta-analysis indicates a strong statistical correlation between limonene (D/L) and α -citral in terms of antioxidant properties. Supported by scientific evidence, studies involving DPPH testing show promising results for EO from dried lemon leaves extracted using hydro-distillation [14, 61]. Similarly, limonene (D/L) has been identified as possessing strong antioxidant properties [25, 27, 67, 68].

Antioxidant assay related to LEO antioxidant activity

The obtained results indicate differences in data representation between various controls and LEO. This disparity is expected due to the differing nature of the controls themselves. Two types of controls were utilized: positive controls, encompassed commercially synthesized antioxidants or isolated compounds with a purity level exceeding 90% [91], and negative controls, which were theoretically devoid of any antioxidant properties; any test results obtained from them would signify instrument errors. Positive controls included substances such as AsA, aspirin, BHT, and quercetin, while the negative control consisted of distilled water. Observations reveal that the majority of positive controls versus LEO yielded negative SMD (Table 2), indicating a statistically lower antioxidant capacity of LEO. Additionally, it was noted that the minimum dosage required to achieve a 50% inhibition of free radicals varied notably, particularly when compared to the significantly large gap seen with pure compounds like AsA. However, in comparison to EO extracted from other plants such as *C. aurantium* and *T. vulgaris*, no significant differences were observed.

When comparing AsA to LEO, direct comparison is impractical due to their inherent differences. LEO consist of diverse mixture of compounds including terpenes, phenols, aldehydes, ketones, alcohols, esters, and other

volatile substances, where the purity of these constituents significantly influences in their antioxidant activity. In contrast, AsA is already in a pure form. However, recent research indicates that its antioxidant capacity is lower compared to BHT, butylated hydroxyanisole (BHA), and trolox [72], attributed to higher proton affinities and transfer enthalpies in both gas and liquid phases [72]. Additionally, studies on a specific variant of limonene derived from lemons [(+)-limonene 1,2-epoxide] suggest that its antioxidant properties are not superior to trolox [92]. Linalool encapsulated with poly (*N*-vinyl) caprolactam demonstrates effectiveness compared to its pure form, although direct comparison with commercial antioxidant compounds [93]. Research on citral indicates that engineering it at a macro level with a complex coordinate of tannic acid-Fe III can augments its efficacy as a radical scavenger [94]. Furthermore, nanoengineering techniques such as nanoemulsions and liposomes have been shown to enhance the biological properties of lemon terpenoids [95, 96].

The relationship between compound measurement and antioxidant assays is closely intertwined. Many antioxidant methods are categorized based on the specific compounds they measure. For instance, the ABTS method measures the ABTS^{•+}. Even indirect tests like TBARS, which measure lipid peroxidation, are considered antioxidant tests because they detect lipid peroxide formation (malondialdehyde or MDA) resulting of oxidation from lipid molecule by OH[•], ROO[•], and O₂^{•-}. As shown in Tables 2 and 3, the antioxidant capacity and IC₅₀ of LEO appear prominently in measuring radicals such as ABTS^{•+}, DPPH[•], H₂O₂, HO[•], and ROO[•]. This is consistent with the categorization of the antioxidant assays depicted in Fig. 3B and 4B. It appears that hydroxyl, peroxy, and peroxide radicals are particularly sensitive to LEO. For example, research indicates that pinene, β -pinene, and limonene can effectively reduce peroxy radicals [90, 97]. Furthermore, there is strong evidence of activity against lipid peroxides such as MDA in the TBARS from limonene [92, 98]. Studies also suggest that linalool, limonene, and sabinene can inhibit protein oxidation caused by MDA and can protect the structure and physicochemical properties of tissue [99].

Conclusions

The meta-analysis identifies plant parts, extraction methods, dominant compounds, and antioxidant assays as key factors influencing the antioxidant properties of LEO. Specifically, lemon leaves and peels display modest IC₅₀, followed by whole plants, residual biomass, and seeds. Maceration emerges as a superior extraction for preserving the antioxidant capacities of LEO

compared to Soxhlet extraction, hydro-distillation, steam-distillation, solvent-free microwave extraction, and microwave-assisted hydro-distillation. Furthermore, limonene, D-limonene, L-limonene, neryl acetate, sabinene, dan z-citral are identified as effective antioxidants (antioxidant capacity and IC₅₀) in LEO, particularly limonene, L-limonene, dan z-citral. LEO demonstrates superior antioxidant capacity compared to essential oil from *C. aurantium*, trolox, dan quercetin. This consistency aligns with significant antioxidant activity observed across multiple assays, including ABTS, β -carotene bleaching, DPPH, and FRAP.

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Author contributions

RB: conceptualization, project administration, funding acquisition, writing-original draft, and review and editing. AK, DNS, and EDL: resource and data curation. TU and PIS: supervision, methodology, validation, writing-original draft, and review and editing. AFMA, DNA, and TW: resource, data curation, investigation, writing-original draft, and review and editing. MMS: conceptualization, supervision, methodology, visualization, and review and editing.

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Data availability

This study utilizes secondary data cited according to the conventions of meta-analysis.

Declarations

Ethical approval and clearance

This research is an original study that has not been submitted nor published elsewhere. It utilizes secondary data sourced from previous research and does not involve studies related to animal welfare or studies involving humans as subjects.

Consent for publication

This research is an original study published exclusively in this journal.

Competing interests

No conflicts of interest are present in this work.

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