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Research Article

Phytochemical analysis and antioxidant evaluation of methanolic extracts from *Lepidium sativum* seeds and *Averrhoa carambola* fruits: A comparative analysis

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Abstract: This study compares the antioxidant potential and phytochemical composition of methanolic extracts from *Lepidium sativum* (seeds) and *Averrhoa carambola* (fruits), chosen due to their traditional medicinal uses and differing plant parts, to explore their potential health benefits. The total phenolic content (TPC), total flavonoid content, and various in vitro antioxidant assays—including 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, hydrogen peroxide scavenging, reducing power, superoxide dismutase (SOD) scavenger, and ferric thiocyanate tests—were evaluated. The results revealed that *Lepidium sativum* exhibited significantly higher TPC (270.34 mg/gm) and flavonoid content (85.05 mg/gm) compared to *Averrhoa carambola* (TPC: 49.04 mg/gm, flavonoids: 21.10 mg/gm). Additionally, *Lepidium sativum* consistently showed superior antioxidant efficacy across all assays, with higher inhibition percentages and lower inhibitory concentration 50 (IC50) values for DPPH, hydrogen peroxide, and superoxide dismutase scavenging. The pronounced antioxidant activity of *Lepidium sativum*, likely due to its enriched phenolic and flavonoid content, highlights its potential as a natural remedy for managing oxidative stress-related diseases and warrants further investigation into its therapeutic applications for oxidative damage management.

1. INTRODUCTION

It is true that many diseases, including diabetes, cancer, cardiovascular conditions, neurological disorders, and autoimmune ailments, are significantly influenced by oxidative stress. An imbalance exists between the body's capacity for neutralizing free radicals (oxygen as well as nitrogen species) through antioxidants and the number of free radicals that are produced (Arika *et al*., 2019). Free radicals are highly reactive molecules generated both internally (endogenously) during normal metabolic processes like respiration and externally (exogenously) from sources like pollution, radiation, and certain medications. These molecules cause damage by oxidizing cellular components like DNA, proteins, and lipids, contributing to disease initiation and progression (Bhat *et al*., 2015; Ulusu *et al*.,2024). The body uses both enzymatic (like glutathione peroxidase, superoxide dismutase, and catalase) and nonenzymatic antioxidants (glutathione, vitamins C and E, and other phytochemicals) as defense mechanisms against oxidative stress. These antioxidants work together to scavenge free radicals and

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maintain a balanced state, thereby safeguarding cellular health (Moriasi *et al*., 2020). Maintaining this equilibrium is crucial for overall well-being. Disruption of this balance, leading to an excess of free radicals and insufficient antioxidant defenses, can result in cellular damage and increase susceptibility to various diseases. Reduced exposure to environmental contaminants, frequent exercise, and a balanced diet high in antioxidants are some lifestyle alterations that can help lower the risks of oxidative stress and its related health issues (Arika *et al.,* 2019). Plants contain antioxidants like flavonoids that fight free radicals, reducing cell damage.

Scientists have been exploring diverse plant compounds to find potent antioxidants for health benefits and potential therapies against oxidative stress-related diseases (Knekt *et al*., 2002; Miller., 1996; Pietta *et al*., 2006). Enzymatic antioxidants like catalase, glutathione peroxidase, and superoxide dismutase, and nonenzymatic ones such as bilirubin, uric acid, and lactoferrin, work to counter free radicals. In diseases, this defense system can be overrun, causing excessive free radicals that damage cells, linked to various illnesses (Vertuani *et al*., 2004). Traditionally, oxidative stress has been tackled using synthetic compounds like butylated hydroxytoluene (BHT), propyl gallate (PG) and butylated hydroxy anisole (BHA). However, these have drawbacks; BHT and BHA, for instance, pose liver risks and can be carcinogenic. Moreover, they're costly and unstable. Given these concerns and the serious impact of oxidative stress, there's a call for safer, more accessible, and potent alternative antioxidants, prompting the current study (Moriasi *et al*., 2020; Mwihia., 2017; Ndhlala *et al*., 2010).

In light of alternative and complementary approaches, medicinal plants have a greater potential of offering effective, secure, reasonably priced, and easily accessible treatments for diseases associated with oxidative stress (Goyal *et al*., 2019). It has been demonstrated that a variety of secondary metabolites present in medicinal plants have a wide range of pharmacological effects. Plants' antioxidative qualities have been shown to protect the body from disease since consuming them reduces the hazard of coronary disease, cancer, high blood pressure, heart attack and memory loss (Ojiewo *et al*., 2013). The main phytochemical groups that contribute to plants' antioxidant potential are polyphenols and vitamins A, C, and E (Moriasi *et al*., 2020). These substances, which include phenols, flavonoids, coumarins, tannins, and anthocyanidins, are generated from benzoic and cinnamic acids and have antioxidant and anticarcinogenic properties (Rajashekar *et al.*, 2009). They serve as vital components in plant defense against various stresses. It is thought that people can receive similar health benefits from consuming plants or items high in these chemicals (Ojiewo *et al*., 2013). Extensive study on nutritional and medicinal plants has been prompted by the search for better substitutes for synthetic antioxidants. The potential of these plants to stop, reverse, or treat oxidative stress-related disorders is being studied (Panche *et al*., 2016).

To that end, we investigated the methanolic extracts' in vitro antioxidant potential obtained from *Lepidium sativum* seeds and *Averrhoa carambola* fruits, considering the phytochemical characteristics of both plants. *Averrhoa carambola*, commonly known as "Kamarakh" or star fruit in India, belongs to the Oxalidaceae family. Native to Malaysia, it is renowned for its versatility and remarkable drought resistance. This tree is a commercial crop in India, valued for its multifaceted uses and edible fruits. While the raw fruit serves as a vegetable, ripened ones find use in various forms like jams, jellies, fermented or unfermented beverages, and desserts (Manda *et al*., 2012; Patil *et al*., 2010; Warrier., 1993). *A. carambola* plant harbors numerous potential medicinal benefits, such as anti-arthritic, hepatoprotective, disinfectant, free-radical scavengers, neuroprotective, and antineoplastic activities. In Ayurvedic and Traditional Chinese Medicine, *A. carambola* is incorporated to address conditions like coughing, dermal fungal infections, eczema, severe headaches, and diarrhea due to its varied therapeutic properties (Patel *et al*., 2015; Wang *et al*., 2016). A few apparent preliminary antioxidant studies of *A. carambola* have been undertaken to assess (Yasmin *et al*., 2013) but a

detailed report on antioxidant efficacy and phytochemical characteristics of the plant is still to be evaluated.

Belonging to the Cruciferae family, *Lepidium sativum*, often known as *L. sativum* Linn., is a tiny annual plant that is widely grown in India. Another name for it is "garden cress." The whole plant is used for ailments such as hemorrhoids and respiratory illness, while the roots are used specifically for tenesmus and the second stage of syphilis (Nadkarni *et al*., 2007). There are numerous impacts associated with its seeds, considered aphrodisiac, galactagogue, and emmenagogue. Boiling the seeds with milk is believed to induce abortion. Additionally, these seeds are beneficial in addressing issues like hiccups, dysentery, and diarrhea (Chopra *et al*., 1992). Scientific investigations have uncovered its potential in reducing hypertension and lowering blood sugar levels (Eddouks *et al*., 2005). Furthermore, studies have explored its impact on fracture healing (Ahsan *et al*., 1989) and its diuretic properties (Wright *et al*., 2007). It has also shown promise in managing bronchial asthma (Paranjape *et al*., 2006) and has been evaluated as a potential oral contraceptive (Sharief *et al*., 2004). A comprehensive and systematic investigation was deemed necessary, despite the execution of preliminary research on the antioxidant properties of the subject (Malar *et al*., 2014; Shirwaikar *et al.,* 2011), employing various in vitro techniques.

2. MATERIALS and METHODS

2.1. Collection of Plant

The Botanical Survey of India (BSI), Koregaon Park, Pune, India provided the plant material that was gathered and authenticated by D.L Shirodkar in the Department of Botany, BSI, WRC PUNE-1, (M.H) India. A voucher specimen of *Averrhoa carambola* L. (SGAC1) and *Lepidium sativum* L. (LSSG1) were preserved in the herbarium of BSI, WRC PUNE-1, (M.H) India.

2.2. Extraction

L. sativum dried and powdered was put in a Soxhlet apparatus thimble. Using a methanol solvent, the extraction was done for eight to ten hours at a temperature of 40 to 60℃ on the heating mantle. Following the extraction procedure, the sample extract was filtered and dried. The extracted products were collected and kept in a sealed container (Alara *et al*., 2019). *A. carambola* methanolic extract of fruit was purchased from the market. The following formula was used to determine the extract's extraction yield:

$$\text{Percentage yield} = \text{Actual yield}/\text{Theoretical yield}*100$$

2.3. Qualitative Analysis of Phytochemicals

Standard procedures for qualitative phytochemical screening were conducted on methanolic extracts of *L. sativum* and *A. carambola* to identify various phytochemical constituents (Harborne., 1998; Kokate., 2006). The assessment relied on visual observation, where the appearance of distinct colors or frothing served as indicators for the presence or absence of specific phytochemical groups.

2.3.1. *Tests for carbohydrates*

In order to find out whether there were any carbs in the extracts, a number of tests were used. First, the extract was subjected to the Molisch test, which required adding strong sulfuric acid and alcoholic α-naphthol solution. This resulted in the formation of a purple ring at the liquid-liquid interface, signifying the presence of carbs. In Fehling's test, which was the second test, the extract was mixed with Fehling's solutions A and B, heated, and formed a brick-red precipitate. The Benedict's test showed color changes (green, yellow, or red) upon boiling equal quantities of Benedict's reagent and extract; these variations indicated the concentration of reducing sugars present. Ultimately, the extract was heated after being mixed with Barfoed's reagent and subjected to Barfoed's test, which produced a red tint indicating the presence of monosaccharides due to the development of cupric oxide.

2.3.2. *Test for alkaloids*

The filtrates from each test extract were subjected to a battery of tests to identify alkaloids following treatment with diluted hydrochloric acid and subsequent filtration. The formation of a white or creamy precipitate indicated the presence of alkaloids in the Mayer's test, which involved adding Mayer's reagent to a portion of the filtrate. Comparably, adding Hager's reagent to a different filtrate part produced a yellow precipitate formation in the Hager's test, which also revealed the presence of alkaloids. Finally, Wagner's test was run by adding Wagner's reagent to a different filtrate. This resulted in the production of a reddish-brown precipitate, which indicated that alkaloids were present in the test extracts.

2.3.3. *Test for flavonoids*

The identification of flavonoids in the extract involved several tests. Firstly, the lead acetate test was conducted by treating the extract with lead (II) acetate solution, potentially resulting in the development of a precipitate that was yellow, a sign that flavonoids were present. Subsequently, sodium hydroxide was added to a portion of the extract for the alkaline reagent test. This produced a strong yellow hue that went neutral when diluted acid was added, suggesting that flavonoids were present. Lastly, the Shinoda test was performed by adding ethanol to the extract, followed by magnesium turning and concentrated HCl. The studied extract included flavonoids as evidenced by the appearance of a pink tint.

2.3.4. *Test for glycosides*

The identification of particular chemicals in the extract was only possible after conducting a series of tests. In order to conduct Borntrager's test, 3 mL of extract was mixed with diluted sulfuric acid, boiled for 300 seconds, and then filtered. Following a vigorous shake of the cold filtrate and adding an equivalent amount of either benzene or chloroform, the organic solvent layer was separated. Anthraquinone glycosides were present in the ammoniacal layer, which turned pink to crimson when ammonia was introduced to it. In Legal's test, however, 1mL of extract was dissolved in pyridine, and then a 10% sodium hydroxide solution was added to make the sodium nitroprusside solution alkaline. A pink to red color indicated the presence of cardiac glycosides. In order to do the Keller-Killiani test, 2 mL of extract with 3ml of glacial acetic acid was mixed. Then, one drop of 5gm of ferric chloride dissolved in 100 mL of water (5%) in a test tube was placed. Subsequently, 0.5 mL of concentrated HCl was cautiously added to the test tube. The acetic acid layer began to turn blue, indicating the presence of cardiac glycosides in the sample under test.

2.3.5. *Test for protein and amino acids*

Tests were performed to identify amino acids and proteins in the sample. In order to do the Biuret test, after heating the sample in 10% sodium hydroxide solution, a drop of 0.7% copper sulphate solution was added. The presence of proteins was indicated by the emergence of a pink or violet tint. The extract was cooked for ten minutes in a water bath containing five percent Ninhydrin solution in order to conduct the Ninhydrin test. The emergence of a blue hue signified the existence of amino acids in the examined sample.

2.3.6. *Test for saponins*

In order to perform the foam test, 1 mL of the plant sample was dissolved in twenty milliliters of distilled water, and the mixture was then forcefully shaken in a graduated cylinder for fifteen minutes. As a result of the test, persistent froth film that was about 1 cm thick was found.

2.3.7. *Test for steroids and triterpenoids*

To determine which particular chemicals were present in the extract, two separate tests were conducted. Salkowski's technique involved treating the plant sample with trichloromethane and then filtering it. Next, conc. HCl was added to the filtrate, agitated, and allowed to settle. Sterols were indicated by a red color in the lower layers, while triterpenes were indicated by a golden

yellow layer at the bottom. However, in Liebermann-Burchard's test, the extract was first treated with trichloromethane, then ethanoic anhydride was added, it was boiled, and then it was cooled. Next, a cautious addition of concentrated HCl was made to the test tube. In the studied extract, the presence of triterpenoids was indicated by the creation of a deep red color while the upper layer turned green. The production of a brown ring at the liquid junction showed the presence of steroids.

2.3.8. *Test for phenolic and tannin chemicals*

To identify the phenolic components in the extract, tests were carried out. For the ferric chloride test, the concentrate was dissolved in purified water, and then a five percent iron chloride solution was added. Phenolic chemicals were present when green, violet or blue colors appeared. In the lead acetate examination, a lead acetate solution was applied to the extract that had been dissolved in distilled water. The presence of phenolic compounds was shown by the production of a white precipitate. In addition, the extract was dissolved in distilled water for the gelatin test, and 1% gelatin solution containing 10% sodium chloride was added. The existence of phenolic chemicals in the extract was shown by the formation of a white precipitate.

2.4. Quantitative Phytochemical Screening

2.4.1. *Quantification of phenolic compounds: Assessing total phenolic content*

The total phenolic component content of the extract was determined using the Folin–Ciocalteu method. 0.5 mL of the extract solution, 2.5 mL of diluted Folin-Ciocalteu reagent, and 1:10 distilled water were combined with 7.5 percent Na_2CO3 w/v. This mixture was incubated in a water bath at 45°C for 30 minutes. Next, at 765 nm, the optical density was determined using a UV-Vis spectrophotometer. To calculate the phenolic content, a typical gallic acid curve was made under the same conditions but with different amounts. The total phenolic component concentration was given in gallic acid equivalents (µg gallic acid equivalent) (Chatoui *et al*., 2020; Djeridane *et al*., 2006; Ulusu., 2023).

2.4.2. *Quantification of flavonoids: Assessing total flavonoid content*

1.25 mL of distilled water was added after 0.075 mL of extract solution and 0.25 mL of sodium nitrite solution were combined in a method using the 5% solution. Five minutes were given to the mixture to sit. After that, 0.15 mL of 10% aluminum chloride was added, and six minutes passed. After that, 0.5 mL of sodium hydroxide (1 M) was added. The combination was diluted with 0.275 milliliters of distilled water, and the 510 nm optical density was then measured. The flavonoid concentration was expressed in quercetin equivalent (µg quercetin equivalent QE/mg extract) using a standard curve created using quercetin (Chatoui *et al*., 2020; Djeridane *et al*., 2006; Quettier-Deleu *et al*., 2007; Ulusu., 2023).

2.5. In-Vitro Antioxidant Assays

2.5.1. *Assessing DPPH scavenging potential*

Using the DPPH test, the ability to neutralize free radicals of the methanolic extract was evaluated in accordance with the methodology outlined by Ali *et al* and Ulusu. Different extract volumes or standard solutions (20–100 µg/mL) were taken out of the stock solution and methanol was added to get the volume down to 1 mL. Each test tube was then filled with 2 mL of 0.1 mM DPPH reagent and thoroughly mixed. Following a half-hour incubation time, the absorbance at 517 nm was determined. The following formula was used to get the percentage inhibitory activity:

$$\% \text{ activity for scavenging} = [(Ac - Ae) / Ac] \times 100$$

In this case, Ac denoted the control's absorbance, while Ae denoted the extract's absorbance. Higher activity in scavenging free radicals was indicated by a lower absorption. Graphs were produced, and linear regression analysis was used to find the half-maximal inhibitory concentration (Ali *et al*., 2013; Ulusu., 2024).

2.5.2. *Assessment of reducing power potential*

Using this procedure, aliquots of the standard and extract (20–100 µg/mL) at different concentrations were combined with 2.5 mL of pH 6.6 phosphate buffer and 2.5 mL of 1% potassium ferricyanide in 1.0 mL of deionized water. After cooling, the mixture was incubated for 20 minutes at 50°C in a water bath. After adding aliquots of 2.5mL of (10%) trichloroacetic acid (TCA) to the mixture, the mixture was centrifuged for 10 minutes at 3000 rpm. A freshly made 0.5 mL (0.1%) ferric chloride solution was combined with 2.5 mL of distilled water to create the upper layer of the solution. The UV spectrometer (Shimadzu-1700) was used to measure the absorbance at 700 nm. Extract was not added to a blank that was prepared. Standard ascorbic acid was utilized at different concentrations ranging from 20 to 100 µg/mL (Choi *et al*., 2002).

2.5.3. *Assessment of superoxide anion radical scavenging capacity*

One milliliter of NBT (nitro blue tetrazolium) in 100 millimolar of a buffered phosphate solution (100 µL of NBT in 100 mM buffered phosphate solution, pH 7.4), one milliliter of NADH (468 microliters in 100 mM buffered phosphate solution, pH 7.4), and different amounts of sample extracts/standard (20, 40, 60, 80, and 100 µg/mL) in methanol were mixed together thoroughly. The reaction was initiated by adding 1 mL of phenazine methosulfate (PMS) prepared as 60 µL of a 100 mM phosphate-buffered solution at pH 7.4. A wavelength spectrometer was used to measure the absorbance at 560 nm after the reaction mixture had been maintained at 30°C for 15 minutes. Simultaneously, an extract-free blank sample was incubated. Ascorbic acid functioned as the reference point for comparing various samples. Higher superoxide anion scavenging activity was indicated by decreased absorbance in the reaction mixture (Nishikimi *et al*., 1972). The calculation of the percentage of scavenge was performed utilizing the following formula: % Inhibition: [(Control absorbance - Sample absorbance) / Control absorbance] x 100.

2.5.4. *Assessing the ferric thiocyanate assay*

This assay indirectly measures the hydroperoxide generated from oxidized linoleic acid during the experimental period. The reaction mixture was put in an Erlenmeyer flask inside a shaker (40°C, 150 rpm) in the dark. It contained 2mL of the sample (or butylated hydroxy toluene/vitamin E as a reference, or methanol as a blank), 2.05 ml of 2.51% linoleic acid in 99.8% ethanol, 4 mL of 0.05 mol/L phosphate buffer (pH 7.0), and 1.95 mL of distilled water. A test tube was filled with 0.1 mL of the reaction mixture, 9.7 milliliters of 75% ethanol, 0.1 mL of 30% ammonium thiocyanate, and 0.1 mL of 2 × 10^2 mol/L ferrous chloride in 3.5% hydrochloric acid in order to measure the antioxidant activity. After three minutes, the absorbance was measured at a wavelength of 500 nM. Every 24 hours, measurements were made until the control's maximum absorbance value was achieved. An unfavorable substitute was made without linoleic acid. BHA and vitamin E were used as positive controls (Ghaima *et al.*, 2013). The following formula was used to calculate antioxidant activity:

$$\% \text{ inhibition} = (Ac - Ae) / Ac \times 100$$

Where Ae is the absorbance of the tested extract samples and Ac is the absorbance of the control reaction.

2.5.5. *Assessment of hydrogen peroxide scavenging potential*

The method developed by Ruch et al. was used to evaluate the extract's ability to absorb hydrogen peroxide (H_2O_2). The extracts, with concentrations ranging from 20 to 100 µg/mL, were measured into 0.1 mL aliquots. To these, 50 mM phosphate buffer at pH 7.4 was added, ensuring the final volume reached 0.4 mL in each Eppendorf tube. Then, 0.6 mL of a hydrogen peroxide solution with a 2 mM concentration was added. Following a 10-minute incubation period, the reaction mixture was agitated, and its absorbance at 230 nm was measured. Serving

as the positive control was ascorbic acid (Ruch *et al.*, 1989). The extract's ability to scavenge hydrogen peroxide was determined using the formula:

% Inhibition: [(Control absorbance - Sample absorbance) / Control absorbance] x 100.

2.6. Statistical Analysis

For n = 6, the results were presented as Mean ± SD. The Bonferroni t-test was used after the one-way analysis of variance (ANOVA) to statistically analyze the results. The significance criterion for group comparisons was set at $p < 0.05$.

3. RESULTS

3.1. Comparative Phytochemical Profiling of Methanolic Extract: A Qualitative Analysis

Phytochemical profiling is crucial for identifying the bioactive compounds in plants, which can lead to the development of new pharmaceuticals and nutraceuticals. The investigation of the methanolic extracts of *L. sativum* and *A. carambola* found the presence of flavonoids, alkaloids, tannins, carbohydrates cardiac glycosides, and saponins. Understanding these compounds' medicinal and nutritional properties validates traditional uses, guides drug development, enhances crop value, and promotes sustainable, natural product use shown in Table 1.

Table 1. Phytochemical profiling of methanolic extract of *L. sativum* and *A. carambola*.

Screening test	*Lepidium sativum*	*Averrhoa carambola*
Glycosides	+	+
Tannins and Phenolic	+	+
Protein and Amino acids	+	-
Terpenoids	+	+
Alkaloids	+	+
Saponins	+	+
Flavonoids	+	+
Protein and Amino acids	+	+
Carbohydrate	-	-
Terpenoids	+	+

(+) present (-) not present

3.1.2 *Estimation of total phenolic content*

The total phenolic content (TPC) of methanol extracts from *L. sativum* and *A. carambola* was measured in terms of gallic acid equivalents (mg/g). The associated data is detailed in Table 2, and Figure 1 illustrates the results graphically.

Table 2. Phytochemical Profiling of Methanolic extract *of L. sativum* and *A. carambola*.

Extracts	*Lepidium sativum*	*Averrhoa carambola*
Absorbance Mean % ± SD*	1.3547 ± 0.003	0.2482 ± 0.002
TPC (mgGAE/g)	270.34	49.04

*Standard deviation *TPC -Total phenolic content

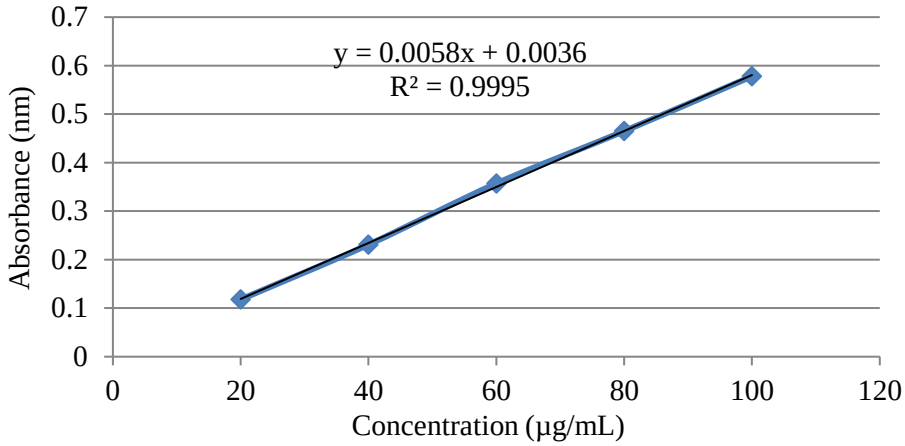

Figure 1. Gallic acid standard curve.

3.1.3. *Estimation of total flavonoid content*

The TFC in *L. sativum* and *A. carambola* methanol extracts was quantified in terms of mg/gm equivalent to gallic acid. The corresponding data can be found in Table 3, and Figure 2 offers a graphical illustration of the results.

Table 3. TFC in *L. sativum* and *A. carambola* methanol extract.

Extracts	*Lepidium sativum*	*Averrhoa carambola*
Absorbance Mean% ± SD*	0.3422 ± 0.002	0.0864 ± 0.004
TFC (mgGAE/g)	85.05	21.10

*Standard deviation , *TFC -Total flavonoid content

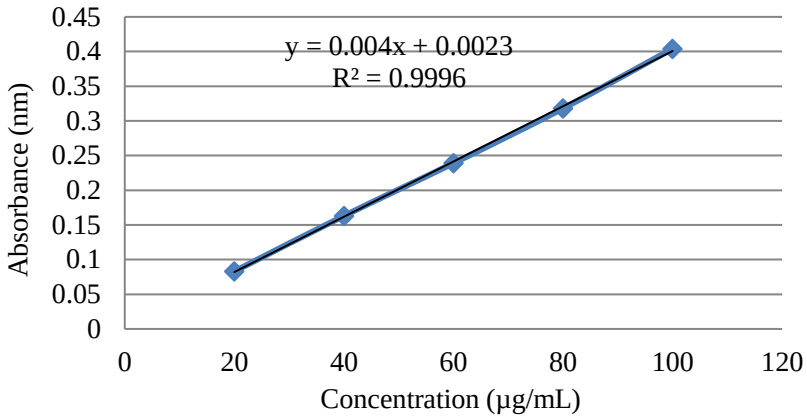

Figure 2. Gallic acid standard curve.

3.2. Assessing in-vitro Antioxidant Potential

Assessing the in-vitro antioxidant potential of methanolic extracts from *Lepidium sativum* (seeds) and *Averrhoa carambola* (fruits) is essential to identify natural sources of antioxidants that neutralize free radicals, potentially preventing chronic diseases like cancer, liver disease and cardiovascular disorders. This evaluation supports traditional medicinal uses, aids in developing new therapeutics, and enhances the nutritional value and market potential of these plants.

3.2.1. *Scavenging of DPPH assay*

At 100 µg/mL, the DPPH radical scavenging activity of *L. sativum* and *A. carambola* methanolic extracts achieved inhibition percentages of 90.569% and 56.165%, respectively, highlighting their varying antioxidant potentials. Their respective IC_{50} values, representing the concentration needed for 50% inhibition, were found to be 33.12 µg/mL for *L. sativum* and

71.041 µg/mL for *A. carambola*. In comparison, ascorbic acid, used as a reference compound, exhibited a higher inhibition percentage of 70.051% and a lower IC_{50} value of 14.013 µg/mL. These findings indicate that although both *L. sativum* and *A. carambola* methanolic extracts possess DPPH radical scavenging activity, their effectiveness is notably lower compared to that of ascorbic acid. The graphical representation can be seen in Figure 3.

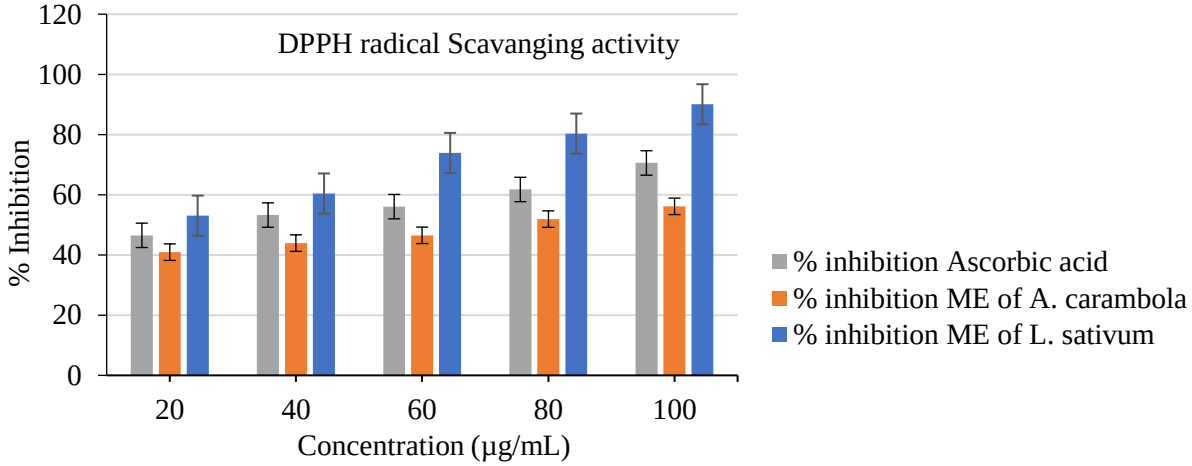

Figure 3. This plot illustrates the DPPH inhibition activity using varying concentrations of ascorbic acid as a standard, in contrast with the methanolic extract of *A. carambola* and *L. sativum*. The inhibition percentages are displayed against the concentrations in (µg/mL), offering a comparative analysis of antioxidant efficacy.

3.2.2. *Hydrogen peroxide (H_2O_2) antioxidant assay*

The methanolic extracts of *L. sativum* and *A. carambola* exhibit significant hydrogen peroxide scavenging activity, with inhibition percentages of 85.185% and 62.139% at 100 µg/mL, respectively, and IC50 values of 39.766 µg/mL for *L. sativum* and 72.54 µg/mL for *A. carambola*. However, ascorbic acid, a well-known antioxidant, shows a higher inhibition percentage of 78.652% and a lower IC50 of 25.021 µg/mL, indicating greater efficacy. These results suggest that while both extracts possess antioxidant properties, their potency is notably lower compared to ascorbic acid, which is more effective at scavenging hydrogen peroxide due to its lower IC50 and higher inhibition efficiency. A graphical representation can be found in Figure 4.

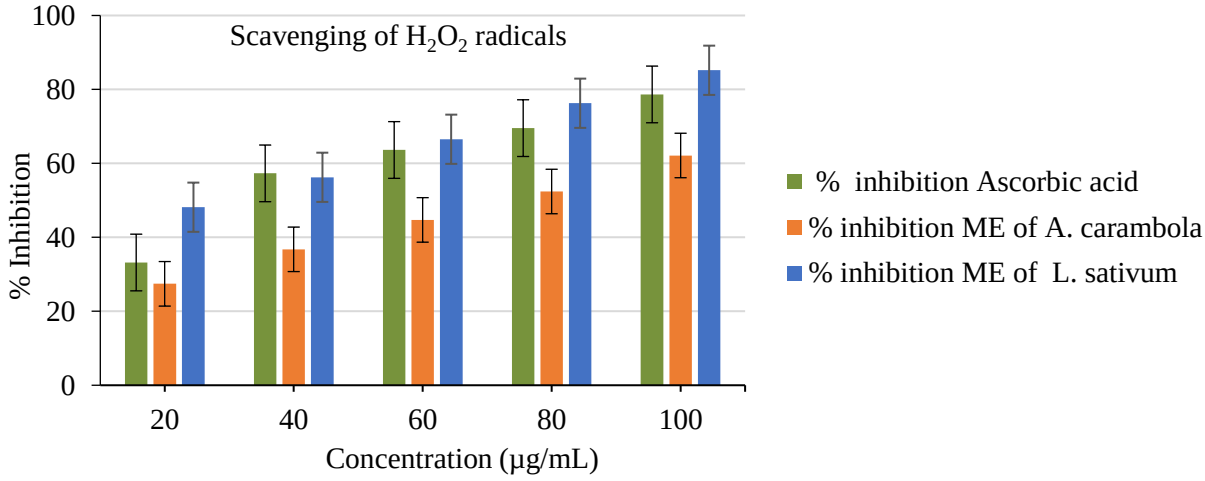

Figure 4. This plot illustrates the H_2O_2 inhibition activity using varying concentrations of ascorbic acid as a standard, in contrast with the methanolic extract of *A. carambola* and *L. sativum*. The inhibition percentages are displayed against the concentrations in (µg/mL), offering a comparative analysis of antioxidant efficacy.

3.2.3. *Ferric thiocyanate antioxidant assay*

At a concentration of 100 µg/mL, the ferric thiocyanate activity of the methanolic extracts from *L. sativum* and *A. carambola* resulted in inhibition percentages of 83.832% and 70.051%, respectively. Their respective IC_{50} values, indicating the concentration required for 50% inhibition, were determined to be 29.52 µg/mL for *L. sativum* and 43.544 µg/mL for *A. carambola*. As a reference compound, ascorbic acid displayed superior inhibitory activity with an inhibition percentage of 62.189% and a notably lower IC_{50} value of 24.606 µg/mL, underscoring its enhanced effectiveness in comparison. These findings suggest that while both *L. sativum* and *A. carambola* methanolic extracts demonstrate ferric thiocyanate activity, their efficacy appears to be slightly lower than that of ascorbic acid. The graphical representation can be seen in Figure 5.

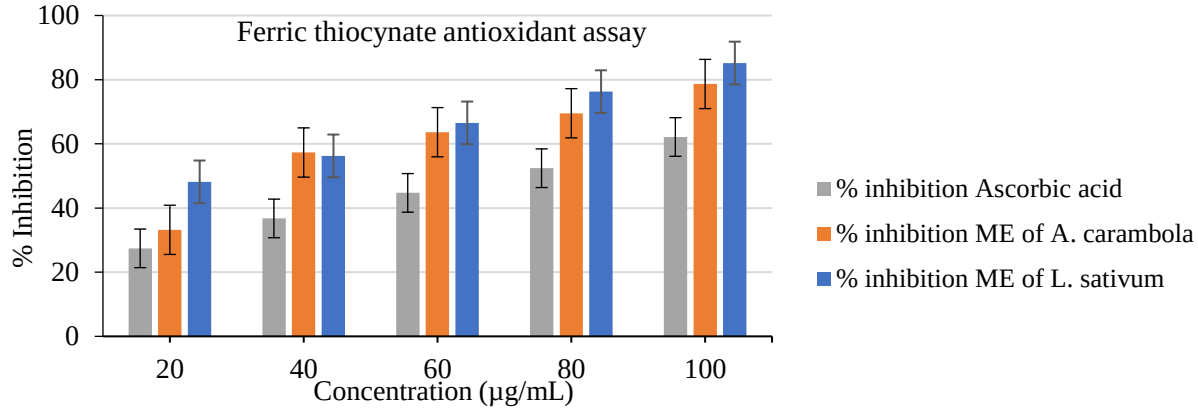

Figure 5. This plot illustrates the ferric thiocyanate inhibition activity using varying concentrations of ascorbic acid as a standard, in contrast with the methanolic extract of *A. carambola* and *L. sativum.* The inhibition percentages are displayed against the concentrations in (µg/mL), offering a comparative analysis of antioxidant efficacy.

3.2.4. *Activity of superoxide to scavenge free radicals*

The SOD scavenger activity of *L. sativum* and *A. carambola* methanolic extracts resulted in inhibition percentages of 85.454% and 78.662%, respectively. Their respective IC_{50} values, representing the concentration required for 50% inhibition, were determined to be 28.595 µg/mL for *L. sativum* and 34.954 µg/mL for *A. carambola*. In comparison, ascorbic acid, acting as a reference compound, exhibited a higher inhibition percentage of 83.864% and a lower IC_{50} value of 10.784 µg/mL. These findings suggest that while both *L. sativum* and *A. carambola* methanolic extracts demonstrate SOD scavenger activity, their effectiveness appears slightly lower compared to that of ascorbic acid, the graphical representation can be seenin Figure 6.

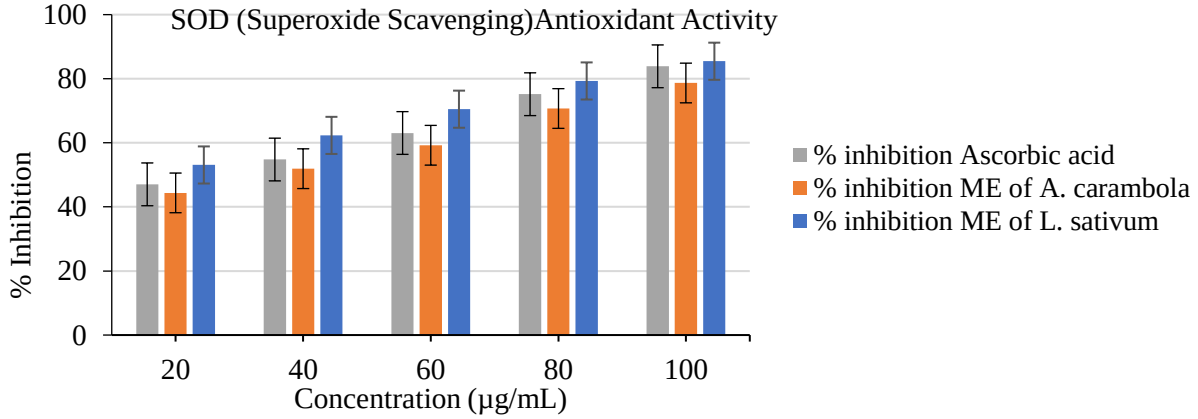

Figure 6. This plot illustrates the superoxide scavenging activity using varying concentrations of ascorbic acid as a standard, in contrast with the methanolic extract of *A. carambola* and *L. sativum*. The inhibition percentages are displayed against the concentrations in (µg/mL), offering a comparative analysis of antioxidant efficacy.

3.2.5. *Ferric reducing antioxidant power (FRAP) assay*

The reducing capacity of a compound is a crucial indicator of its potential antioxidant activity. Ascorbic acid, a dietary antioxidant, was employed for comparison. Compounds demonstrating high reducing power serve as electron donors, capable of reducing oxidized intermediates in lipid peroxidation processes. This ability enables them to function as primary and secondary antioxidants. In the comparison between the methanolic extracts of *L. sativum* and *A. carambola*, *L. sativum* exhibited notably strong reducing capacity. This implies that *L. sativum* extract possesses a higher ability to donate electrons, potentially making it more effective in combating oxidative stress and protecting against lipid peroxidation compared to *A. carambola* extract.The graphical representation are shown in Figure 7.

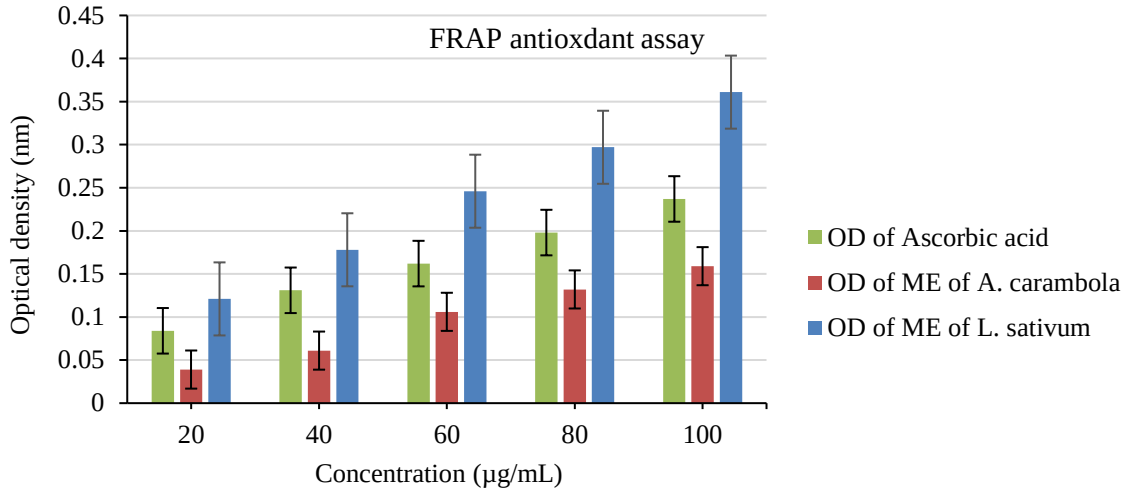

Figure 7. This plot illustrates the superoxide scavenging activity using varying concentrations of ascorbic acid as a standard, in contrast with the methanolic extract of *A. carambola* and *L. sativum*. The inhibition percentages are displayed against the concentrations in (µg/mL), offering a comparative analysis of antioxidant efficacy.

4. DISCUSSION

The methanol extract from *L. sativum* stands out with significantly higher concentrations of both total phenolic content (TPC) and total flavonoid content (TFC) compared to *A. carambola*. These findings indicate that *L. sativum* harbors higher concentrations of beneficial phenolic and flavonoid compounds, suggesting superior antioxidant potential and therapeutic benefits compared to *A. carambola*. Antioxidants are pivotal in shielding our body against diseases by mitigating oxidative damage caused by reactive oxygen species (ROS) to cellular components. Recent studies highlight the therapeutic potential of plant-based antioxidants possessing free-radical scavenging properties in combating free radical-related ailments such as obesity, diabetes, cancer, digestive problems, rheumatoid arthritis, brain disorders, coronary heart disease, and ageing naturally (Tan *et al*., 2018). Methanolic extracts are used to evaluate antioxidant activity from *L. sativum* and *A. carambola* were scrutinized across various parameters. *L. sativum* consistently emerged as a potent antioxidant, showcasing higher inhibition percentages and lower IC_{50} values compared to *A. carambola* in scavenging DPPH radicals, neutralizing hydrogen peroxide, and acting as an SOD scavenger. Moreover, *L. sativum* exhibited a superior reducing capacity, indicating its potential as an effective electron donor and antioxidant agent. While both extracts displayed antioxidant properties, *L. sativum* demonstrated more robust efficacy across multiple assays, albeit falling short of the antioxidant prowess exhibited by the standard, ascorbic acid.

5. CONCLUSION

In summary, the study highlights *L. sativum* as a potent antioxidant compared to *A. carambola*. With higher concentrations of beneficial compounds like phenolics and flavonoids, *L. sativum*

demonstrates superior antioxidant potential. It shows stronger efficacy in various tests, indicating its promise as an effective natural antioxidant, although not surpassing the standard, ascorbic acid. The present findings suggest that L. sativum has potential for therapeutic applications, particularly in combating diseases linked to oxidative damage.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Suchita Gupta: Investigation, Resources, Visualization, Software, Formal Analysis, and Writing -original draft. **Reena Gupta**: Methodology, Supervision, and Validation. Authors may edit this part based on their case.

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