ijlpr 2023; doi 10.22376/ijlpr.2023.13.4.P1-P13

International Journal of Life science and Pharma Research

ISSN 2250-0480

Research Article

Antioxidant Activity of Usitatissimum and Mentha Spicata

Synergistic in Vitro Antioxidant Activity of *Linum Usitatissimum* and *Mentha Spicata*

Dr.T. Ashwini[1], Dr. Arul Amutha Elizabeth[2] and Dr.Ramya Ravichandar[3*]

[1]*Postgraduate, Department of Pharmacology, Sree Balaji Medical College & Hospital, Chrompet, Chennai, Tamilnadu, India PIN- 600044.*

[2]*Professor&Head, Department of Pharmacology, Sree Balaji Medical College & Hospital, Chrompet, Chennai, Tamilnadu, India.PIN- 600044.*

[3*]*Associate Professor, Department of Pharmacology, Sree Balaji Medical College & Hospital, Chrompet, Chennai -600044, Tamilnadu, India.PIN- 600044.*

Abstract: Reactive Oxygen Species (ROS) are free radicals that contain oxygen and easily react with another cell molecule. Excessive ROS formation damages the macromolecules of a cell, like lipids, DNA, and proteins, leading to oxidative stress. Oxidative stress occurs when there is an imbalance between free radical production and the body's ability to neutralize them through anti-oxidants. Oxidative stress can lead to atherosclerosis, Parkinson's disease, rheumatoid arthritis, diabetes, cancer, etc. Synthetic anti-oxidants, such as Butylated hydroxyanisole (BHA), have recently been allegedly dangerous for human health. Thus, the need arises for searching and identifying natural sources of antioxidants, which would be useful for the primary prevention of the above disorders. The main objective of this study is to explore the synergistic in vitro anti-oxidant activity of flaxseed (*Linum usitatissimum*) and mint (*Mentha spicata*) combination. It was achieved by performing in vitro spectrophotometric techniques such as ABTS (2,2-azinobis-(3- ethylbenzothiazoline-6-sulfonic acid)) and DPPH(2,2-Diphenyl-1-picrylhydrazyl) H_2O_2(hydrogen peroxide) and hydroxyl radical scavenging assays. The percentage of inhibition was calculated, and the results were expressed in terms of IC_{50} (half maximal inhibitory concentration). Although there are numerous studies on the antioxidant properties of *Linum usitatissimum* and *Mentha spicata*, there are no reports on their comparative and synergistic antioxidant properties. This study was therefore performed to investigate the comparative and synergistic anti-oxidant properties of *Linum usitatissimum* and *Mentha spicata*.

Keywords: Anti-oxidant, Synergistic, Flaxseed, Mint, DPPH, ABTS.

***Corresponding Author**

Dr.Ramya Ravichandar , Associate Professor, Department of Pharmacology, Sree Balaji Medical College & Hospital, Chrompet, Chennai -600044, Tamilnadu, India.PIN- 600044.

Received On 19 September, 2022
Revised On 16 February, 2023
Accepted On 2 March, 2023
Published On 1 July, 2023

Funding This research did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors.

Citation Dr.T. Ashwini, Dr. Arul Amutha Elizabeth and Dr.Ramya Ravichandar , Synergistic in Vitro Antioxidant Activity of Linum Usitatissimum and Mentha Spicata.(2023).Int. J. Life Sci. Pharma Res.13(4), P1-P13 http://dx.doi.org/10.22376/ijlpr.2023.13.4.P1-P13

1. INTRODUCTION

Free Radicals are molecules or atoms with an unpaired electron in their outermost shell, because of which they are highly reactive and unstable. ROS are free radicals containing oxygen that easily react with another cell molecule.[1] Free radicals in the body can be produced from endogenous and exogenous sources.[2] The major endogenous free radicals are produced from a cell's metabolism in mitochondria. In the Electron Transport Chain, where oxygen gets reduced to water to generate energy, few oxygen molecules get converted to superoxide anion, a reactive oxygen species. Superoxide anion leads to the development of other reactive oxygen species like hydroxyl radical and hydrogen peroxide.[3] Exogenous free radicals can be from UV light, air pollution, smoking, ionizing radiation, etc. Every cell in the body can produce ROS, which gets neutralized by endogenous anti-oxidants like catalase, glutathione peroxidase, and superoxide dismutase. When there is excessive formation of ROS, it damages the macromolecules of a cell, like lipids, DNA, and proteins, inducing oxidative stress. [1] Oxidative stress is an imbalance between the production of ROS and the body's ability to neutralize them through anti-oxidants. It affects various organs in the body, mainly causing atherosclerosis and inflammatory disorders like neurodegenerative disorders like Parkinson's disease, rheumatoid arthritis, aging, cancer, etc.[4]So; it is important to neutralize the excessive ROS using anti-oxidants exogenously. Exogenous anti-oxidants can be classified into natural and synthetic anti-oxidants based on the source. Anti-oxidants can also be categorized as primary or secondary anti-oxidants based on the mechanism. They are called so because primary anti-oxidants stabilize the free radicals directly by single electron transfer or donating a hydrogen atom. Secondary antioxidants inhibit the pathways that produce oxidant species. Antioxidants may also be incorporated into food products to be used as preservatives for storage and transportation.[5]Synthetic antioxidants include Butylated hydroxyanisole (BHA), Butylated hydroxytoluene (BHT), Butylated hydroxyanisole (BHA), propyl gallate (PG) and tert-butyl hydroquinone (TBHQ). Synthetic antioxidants have recently been alleged to be dangerous for human health, possessing carcinogenicity.[6] Thus, there arises the need for the search for nontoxic natural compounds with antioxidant effects. Natural antioxidants offer low-cost advantages, good diet compatibility, and a good safety profile. Hence, identifying natural sources of antioxidants and their addition to lifestyle would be useful for the primary prevention of the above disorders. Various natural products, such as fruits, vegetables, tea, etc., have been extensively researched for the presence of antioxidant compounds.[7,8,9]

1.1. Chemical constituents and antioxidant components of Linum usitatissimum and Mentha spicata and their antioxidant activities

Linum usitatissimum is commonly known as flaxseed. The major chemical components of *Linum usitatissimum* are flavonoids, phenolic acids, lignans (secoisol lariciresinol di-glucosides- SDG), tannins, and phenylpropanoids. 10 Apart from these, flaxseed possesses alpha-linolenic acid, which produces anti-inflammatory action, and dietary fiber, which lowers cholesterol levels. These add up to its beneficial role on the cardiovascular system. 11 Thus, extracts from *Linum usitatissimum* exhibit considerable antioxidant, anti-inflammatory, anti-microbial, anti-cancer, anti-diabetic, and cognitive effects.[12] *Mentha spicata*, spearmint or mint, is a common household herb. Spearmint contains lignans, polyphenolic and flavonoid compounds which have anti-oxidant activity.[13]Polyphenols are a major group of compounds that play a part in the antioxidant effect of *Mentha spicata* owing to their free radical scavenging capability due to their hydroxyl groups.[14]The essential oil extracted from Mentha has been used traditionally for treating cold, bronchitis, and sinusitis. Mint is commonly used for treating indigestion and abdominal pain.[15]

1.2. Mechanism of antioxidant action of Linum usitatissimum and Mentha spicata

The antioxidant activity of recognized antioxidants has been ascribed to various mechanisms such as prevention of chain initiation, transition metal ion catalyst binding, radical scavenging, and reductive capacity. The anti-oxidant capacity of flaxseed has been analyzed in both in vitro and in vivo models. Flaxseed exerts antioxidant potential using various mechanisms. SDG contained in flaxseed shows antioxidant activity by inhibition of lipid peroxidation. 16 The anti-oxidant components of flaxseed decrease ROS production and augment ROS elimination. In a concentration-dependent manner, flaxseed inhibits the lipid peroxidation of liver homogenate. It also brings down the elevated hepatic enzymes to normal levels.[17] Spearmint (*Mentha spicata*) has polyphenols which are phenolic components known to exert potent anti-oxidant activity by various mechanisms such as transition metal chelation, hydrogen atom transfer, and single electron transfer.[18]

1.3. Factors influencing the antioxidant capacity of Linum usitatissimum and Mentha spicata

The most powerful antioxidant components present in flaxseed extracts are phenolics and flavonoids. Different anti-oxidant methods are used to test and assess the anti-oxidant capacity of compounds extracted with different polarity solvents. The highest amount of flavonoids is detected by extraction with 80% ethanol, indicating the highest antioxidant capacity.80% aqueous ethanol and pure methanol are the most effective solvents for demonstrating the antioxidant potential of flaxseed.[19] Rosmarinic acid is a polyphenolic compound that contributes to the antioxidant potential of *Mentha spicata*. Flowering leads to the reduction of rosmarinic acid levels in *Mentha spicata*. So, plants must be harvested before flowering. Day length is an important environmental factor influencing the antioxidant potential of *Mentha spicata*. To maximize rosmarinic acid accumulation, they must be grown in areas with long days exceeding 14 hours.[20]It was observed that higher phenolic compounds were found in those cultivated in open fields than those cultivated under greenhouse condition.[21]Various studies were performed to determine the influence of agro-climatic conditions on the antioxidant capacity of *Mentha spicata*. They proved that the plants raised at higher altitudes exhibited better antioxidant potential than those cultivated in the plains.[22]

1.4. Ingredient synergism

Synergism implies that when two or more herbal ingredients are combined, they mutually augment each other's effect more than the simple summation of these ingredients.[23] Flaxseed (*Linum usitatissimum*) produces gastric adverse effects such as nausea, bloating, and indigestion. [24] These can

be combated by combining with mint (*Mentha spicata*). In a study by Mehraban et al., a combination of flaxseed and spearmint extract improved the endocrine profile and the histomorphology of the ovary in PCOS.[25] Thus, a combination of flaxseed and spearmint extract finds clinical usefulness in various disorders. Numerous studies proved their antioxidant activity. Still, there need to be more studies to prove the synergistic free radical scavenging effect of *Linum usitatissimum* and *Mentha spicata*. Hence, this study aims to explore the synergistic *in vitro* anti-oxidant activity of flaxseed extract and mint extract as a combination.

2. MATERIALS AND METHODS

There are various methods available for the assessment of the antioxidant capacity of a compound. In our study, the antioxidant effect of the combination of *Linum usitatissimum* and *Mentha spicata* was evaluated using ABTS assay, DPPH assay, FRAP assay, hydroxyl radical scavenging assay, and hydrogen peroxide assay.[26]

2.1. Preparation of Extract

Mint leaves were taken, dried, and ground into fine powder. Flaxseeds were taken, dried, and ground into fine powder. 50g of flaxseed powder, 50g of mint leaf powder, and 50 gm of combined powder of mint leaves and flax seed were added and placed in separate clean conical flasks. 250ml of methanol was added to all three conical flasks. The methanolic extract was prepared following the method of Oraemesi I.[27]Accordingly; the extraction was carried out in the Soxhlet apparatus for 72 hours. The Solvent was evaporated using a distilled water bath, and the extract was stored at 4 degrees Celsius for further use. 1mg/ml of prepared extract was taken, and this was serially diluted to get different concentrations (1.5-1000 µg/ml).

2.2. DPPH Assay

DPPH(2,2-Diphenyl-1-picrylhydrazyl) is a stable free radical that is purple and readily accepts hydrogen atoms from anti-oxidants when combined and gets converted to 2,2-Diphenyl-1-picrylhydrazyl which is yellow. Ferulic acid is known to have the strongest anti-oxidant activity and has been used as the standard in many *in vitro* antioxidant studies. Hence, ferulic acid was used as standard in the DPPH assay.[28] 1mg of ferulic acid (standard) was weighed and dissolved in methanolic Solvent to prepare 1mg/ml concentration. It was serially diluted to obtain different concentrations (1.5-1000 µg/ml) similar to the sample. Since the standard and sample were prepared in methanol solvent, DPPH was prepared by dissolving 0.1mM in 100% methanol. About 100µl of each concentration from the sample was taken and added to the test tubes. About 100µl of the standard was added to a test tube. 900µl of DPPH was added to each well. The control blank contained DPPH and Solvent without extract. Sample blank was also prepared without DPPH. After vortexing, the sample, standard, and control were incubated for 30 minutes at room temperature. After incubation, absorbance was measured at 517nm. The experiment was repeated in triplicates, and the percentage of inhibition was calculated using the formula below.[29]

$$\% \text{ of inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of test})}{\text{Absorbance of control}} \times 100$$

2.3. ABTS Assay

ABTS's incubation with potassium persulfate oxidizes, forming the radical 2,2-azinobis-(3- ethylbenzothiazoline-6-sulfonic acid) (ABTS•+). This free radical was reduced in the presence of hydrogen-donating antioxidants, and the reduction was measured spectrophotometrically at 734 nm. The working solution was prepared by adding 7.4mM ABTS and 2.6 mM potassium persulphate in equal volume (1:1 v/v). This working solution was incubated in the dark for 16 hours at room temperature. Then the solution was diluted with methanol until the absorbance reached 0.706±0.001 at 734 nm. Finally, 100µl of all the samples were added to 3.9ml of ABTS•+, and the absorbance was measured at 734 nm after six minutes. Ascorbic acid is well known to possess the strong anti-oxidant activity and is used as the standard in many *in vitro* antioxidant studies. Therefore, ascorbic acid was used as the standard.[30] Ascorbic acids was used as the standard. First, 1mg of ascorbic acid (standard) was weighed and dissolved in a methanolic Solvent to prepare a 1mg/ml concentration. Then, it was serially diluted to obtain different concentrations (1.5-1000 µg/ml) similar to the sample. All the experiments were done in triplicates.[31] Percentage of inhibition was calculated using the formula below.

$$\% \text{ of inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of test})}{\text{Absorbance of control}} \times 100$$

2.4. Ferric Reducing Antioxidant Power Assay –FRAP

Ferric reducing antioxidant power assay (FRAP) was measured similarly to the procedure described by Iris et al., 1996.[32] The FRAP reagent contained 300mM of acetate buffer with a pH 3.6,10mM of TPTZ(2,4,6-tri(2-pyridyl)-s-triazine) in 40mM dilute HCl and 20mM of Ferric chloride. The FRAP reagent was prepared by mixing the above constituents in the ratio of 10:1:1. FRAP assay utilizes antioxidants as reducing agents in a redox-linked colorimetric method. Reduction of ferric tri-pyridyl triazine (Fe III TPTZ) complex to ferrous form (which produces intense blue color) occurs at low pH and can be observed by measuring the change in absorption at 593 nm. Hence, the change in absorbance indicates the total reducing power of the antioxidants in the reaction mixture. Various concentrations of the samples were aliquoted and made up to 1 ml with distilled water and mixed with 3 ml of working FRAP reagent, and incubated at 37°C for 30 minutes. After incubation, the absorbance was measured at 593 nm. The ferrous sulfate standard was processed by the same procedure using various concentrations of ferrous sulfate. Blank consisted of all the reagents except for the extract or standard solution, which was substituted with water.

2.5. *Hydrogen peroxide (H_2O_2) assay*

A hydrogen peroxide (H2O2) assay was performed based on the procedure described by Kowsalya et al., 2014. [33]An aliquots of 50 mM H_2O_2 and different concentrations of samples and standards were mixed. To this, 10 µl of methanol and 900 µl of FOX reagent were added. This mixture was incubated for 30 min at room temperature. After incubation, 90 µL of the H_2O_2-sample solution was mixed with 10 µL HPLC-grade methanol, and 0.9 mL FOX reagent was added (4.4 mM BHT added in 9 volumes of Methanol and 1 volume of 1 mM xylenol Orange, 2.56 mM Ammonium ferrous sulfate in 0.25 M H_2SO_4). The reaction mixture was then incubated at room temperature for 30 minutes. The absorbance was measured at 560 nm. The percentage of inhibition was calculated as follows:

$$\% \text{ of inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of test})}{\text{Absorbance of control}} \times 100$$

2.6. *Hydroxyl radical scavenging assay*

Hydroxyl radical scavenging assay was assessed according to the method of Ramakrishna et al., 2012.[34]The reaction mixture containing 0.2ml of $FeCl_3$ (100 µM), 0.2ml of EDTA (1.04 mM), 100µl of H_2O_2 (1 mM) and 2-deoxy- D-ribose (2.8 mM) in 20mM KOH-KH2PO4 buffer (100 µl) was added to various concentrations of the sample. These were incubated for 1 hour at 37°C. After incubation, 1ml of 0.5% Thiobarbituric acid and 2.8% Trichloroacetic acid were added to the mixtures, which were incubated for 15 minutes at 95°C. Ascorbic acid was used as the standard. The absorbance of the resultant solution was measured at 532 nm. The percentage of inhibition was calculated as follows:

$$\% \text{ of Inhibition} = \frac{\text{Blank - Test}}{\text{Blank}} \times 100$$

3. RESULTS AND DISCUSSION

Flaxseed (*Linum usitatissimum*) has many important substances, among which lignan is the major component exerting its anti-oxidant activity by hydroxyl radical scavenging mechanism.[35] Spearmint (*Mentha spicata*) has polyphenols which are phenolic components known to exert potent anti-oxidant activity by various mechanisms such as transition metal chelation, hydrogen atom transfers, and single electron transfer. Although studies are available on the antioxidant properties of *Linum usitatissimum* and *Mentha spicata*, there are no reports on their comparative and synergistic antioxidant properties. This study was therefore performed to investigate the comparative and synergistic antioxidative properties of *Linum usitatissimum* and *Mentha spicata*. *In vitro*radical scavenging activity of *Linum usitatissimum* and *Mentha spicata* was assessed by the ability to scavenge DPPH, ABTS, Reductive Ability, Hydrogen Peroxide, and Hydroxyl Radicals. Assessment of primary antioxidant activity was performed using the stable radical DPPH. Both *Linum usitatissimum* and *Mentha spicata* exhibited DPPH radical scavenging activity. The percentage of inhibition was calculated, and the results were expressed in terms of IC_{50}. IC_{50} is the half maximal inhibitory concentration, the substance concentration required to scavenge the radical by 50%.[36] In our study, the scavenging activity of *Linum usitatissimum* extract measured as the DPPH radical activity significantly varied among different concentrations ranging from 10 to 100 µg/ml. The *Linum usitatissimum* extract showed concentration-dependent anti-oxidant activity with a maximum 71.5 % inhibition at 100µg/ml of sample (*Linum usitatissimum*) concentration, as shown in Table 1, and IC50 was found to be 66.7µg/ml. The maximum DPPH scavenging rate obtained by Liang et al. was found to be 58.2% at a concentration of 2mg/ml.[37]According to the study by Deme et al., the maximum scavenging rate was78% at a concentration of 10 mg/ml.[38]In the study by Amin et al., the maximum inhibition produced was 82.53% at a concentration of 50 mg/ml.[39] Chera et al. showed that *Linum usitatissimum* extract possessed antioxidant activity with an IC_{50} value of 39.07 ± 2.84 by DPPH assay. [40] In the study by Han H et al., the lignan extract of flaxseed (*Linum usitatissimum*) exhibited concentration-dependent anti-oxidant activity with an IC50 value of 53.30µg/ml by DPPH assay.[41] In our study, the scavenging activity of *Mentha spicata* extract measured as the DPPH radical activity varied significantly among different concentrations ranging from 10 to 100 µg/ml. The *Mentha spicata* extract showed concentration-dependent anti-oxidant activity with a maximum 82.8 % inhibition at 100µg/ml of sample (*Mentha spicata*) concentration, as shown in Table 2, and IC50 was found to be 52.3 µg/ml. Diethyl ether extract of *Mentha spicata* showed almost 100% antioxidant activity at 40µg/l by DPPH assay according to the study by Choudhury et al.[42]In a study done by El Menyiy et al., the essential oil extracted from *Mentha spicata* exhibited potent free radical scavenging activity, with IC_{50} value of 5.96 by DPPH assay.[43]*Mentha spicata* leaves produced IC_{50} of 10 ± 0.24 µg/mL by DPPH assay according to the study performed by Dhifi et al.[44]Selles et al. proved that *Mentha spicata* leaves produced significant antioxidant activity with IC_{50} of 21.19 ± 7.17 µg/mL by DPPH assay.[45]According to the study by Brahmi et al., the essential oil from the leaves of *Mentha spicata* showed IC_{50} of 9544.6 ± 196.2 µg/mL by DPPH assay.[46] In our study, based on the DPPH assay, the standard (ferulic acid extract) showed maximum inhibition of 91.1% at 100µg/ml, and IC50 was found to be 45.7µg/ml. Concentration-dependent antioxidant activity with 93.2% inhibition was exhibited at 100µg/ml by the combination of *Linum usitatissimum* and *Mentha spicata*, and IC50 of the combination was found to be 27.3 µg/ml. (Figure 1, Table 3, and Table 4).

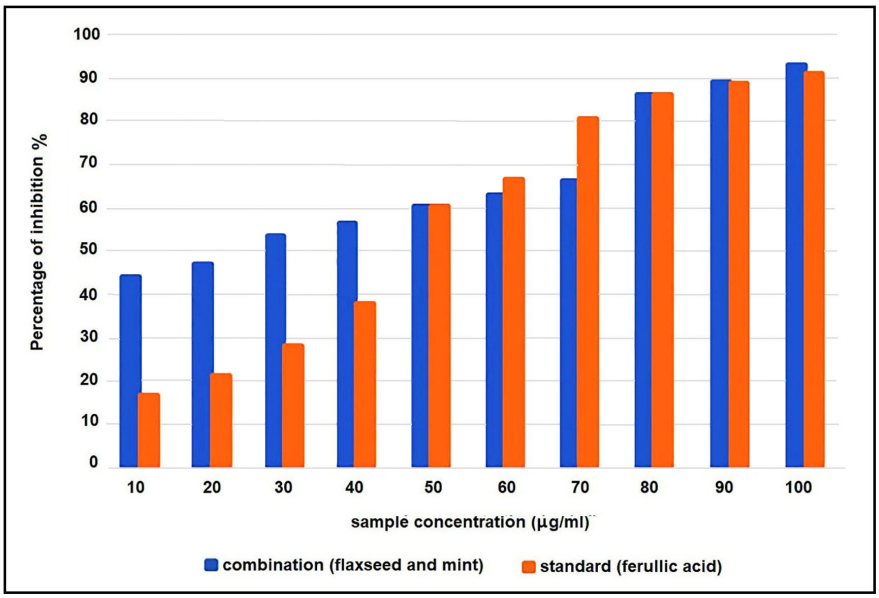

Fig 1: shows the percentage of inhibition of combination (*Linum usitatissimum* and *Mentha spicata*) extracts at different concentrations and standard ferulic acid by DPPH assay.

Table 1: *Linum usitatissimum*- Sample Concentration and Their Corresponding Percentage of Inhibition at Varying Concentration Using DPPH Assay

Sample concentration (µ g/ml)	10	20	30	40	50	60	70	80	90	100
% of inhibition	9.1	14.8	27.5	32.3	38.9	44.2	52.3	58.7	62.8	71.5

Table 2: *Mentha Spicata*- Sample Concentration and Their Corresponding Percentage of Inhibition at Varying Concentration Using DPPH Assay

Sample concentration (µ g/ml)	10	20	30	40	50	60	70	80	90	100
% of inhibition	18.2	24.3	34.1	39.4	50.8	54.7	62.5	69.1	75.7	82.8

Table 3: Standard (Ferulic Acid)- Sample Concentration and Their Corresponding Percentage of Inhibition at Varying Concentration Using DPPH Assay

Sample concentration (µ g/ml)	10	20	30	40	50	60	70	80	90	100
% of inhibition	16.8	21.4	28.4	38.2	60.5	66.9	80.7	86.2	88.9	91.1

Table 4: Combination (*Linum usitatissimum* and *Mentha Spicata*)- Sample Concentration and Their Corresponding Percentage of Inhibition at Varying Concentration Using DPPH Assay

Sample concentration (µ g/ml)	10	20	30	40	50	60	70	80	90	100
% of inhibition	44.3	47.09	53.8	56.8	60.5	63.3	66.6	86.2	89.2	93.2

In our study, the DPPH assay showed that at a maximum concentration of 100µg/ml, the percentage of inhibition produced by *Linum usitatissimum*, *Mentha spicata*, and the combination is 71.5%, 82.8%, 93.2%, respectively, as depicted in Figure 2. Therefore, by comparing, we conclude that the combination possesses higher antioxidant activity than individual extracts, thus showing synergism.

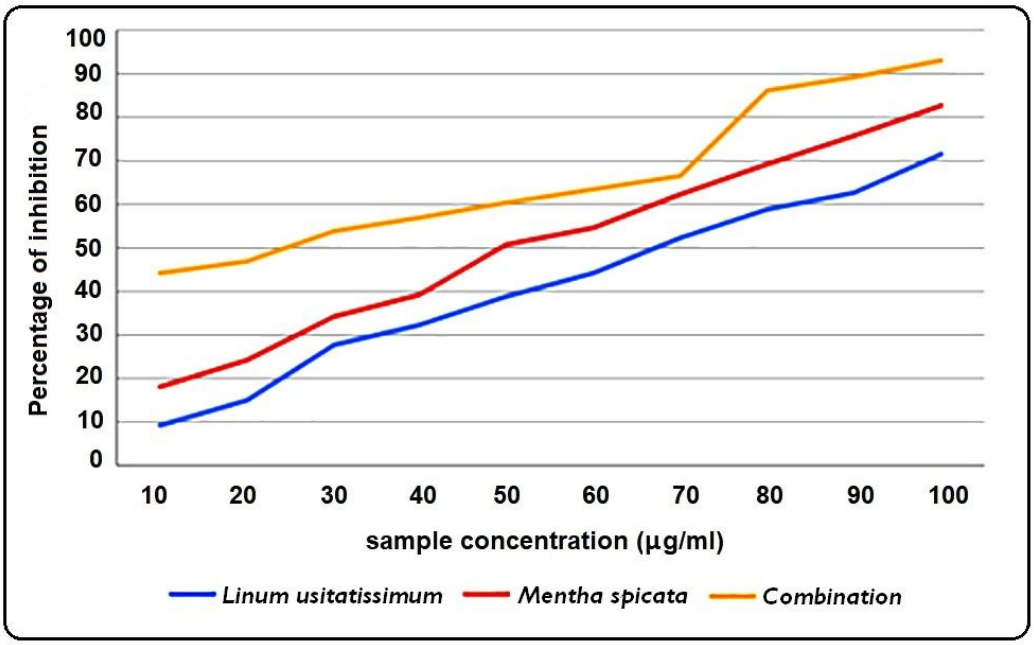

Fig 2: Shows the percentage of inhibition of extracts of *Linum usitatissimum*, *Mentha spicata*, and the combination at different concentrations by DPPH assay.

The free radical scavenging activity of *Linum usitatissimum* and *Mentha spicata* was tested using the ABTS method. In our study, the scavenging activity of *Linum usitatissimum* extract measured by the ABTS assay varied significantly among different concentrations ranging from 10 to 100 µg/ml. The *Linum usitatissimum* extract showed concentration-dependent anti-oxidant activity with a maximum of 68.6 % inhibition at 100µg/ml of sample (*Linum usitatissimum*) concentration, as shown in Table 5, and IC50 was found to be 70.7µg/ml. A study by Safdar et al. showed that at a concentration of 30 mg/ml, flaxseed gum exhibited a percentage of inhibition of 72.39 ± 1.30 by ABTS assay, which was the maximum antioxidant activity of the compound.[47]Han H et al. demonstrated that the lignan extract of flaxseed(*Linum usitatissimum*) exhibited concentration-dependent anti-oxidant activity with IC50 value of 27.72µg/ml by ABTS assay.[41]Liang et al. found that *Linum usitatissimum* exhibited ABTS radical scavenging rate of 91.2% at 25 mg/ml.[48]Bouaziz et al. demonstrated that at a higher concentration of 40 mg/ml, the percentage of inhibition produced by flaxseed gum was 75.6% by scavenging ABTS radicals.[49] Based on ABTS assay, the *Mentha spicata* extract showed concentration-dependent anti-oxidant activity with a maximum 82% inhibition at 100µg/ml of sample (*Mentha spicata*) concentration as shown in Table 6, and IC50 was found to be 53.7 µg/ml. *Mentha spicata* leaves produced IC_{50} of 10.3 ± 0.9 µg/mL by ABTS assay according to the study performed by Fatiha et al.[50]Nickavar et al. established that aerial parts of *Mentha spicata* (ethanolic extract) showed $IC_{50}$of 173.80 µg/mL by the ABTS assay.[51]According to the study by Brahmi et al., the essential oil from the leaves of *Mentha spicata* showed IC_{50} of 36.2 ± 3.2 µg/mL by ABTS assay.[46] Arumugam et al. demonstrated that ethyl acetate extract of *Mentha spicata* showed maximum antioxidant activity of 95% at 20 µg/ml by ABTS assay.[52]Among these, all the studies except Nickavar et al. showed lesser IC_{50} when compared with our study. In our study, the ABTS assay of the standard (Ascorbic acid extract) showed maximum inhibition of 87.4% at 100µg/ml, and IC_{50} was found to be 51.7µg/ml. Concentration-dependent antioxidant activity with 87% inhibition was exhibited at 100µg/ml by *Linum usitatissimum* and *Mentha spicata* combination, and IC_{50} of the combination was found to be 52.1 µg/ml. (Figure 3, Table 7, and Table 8).

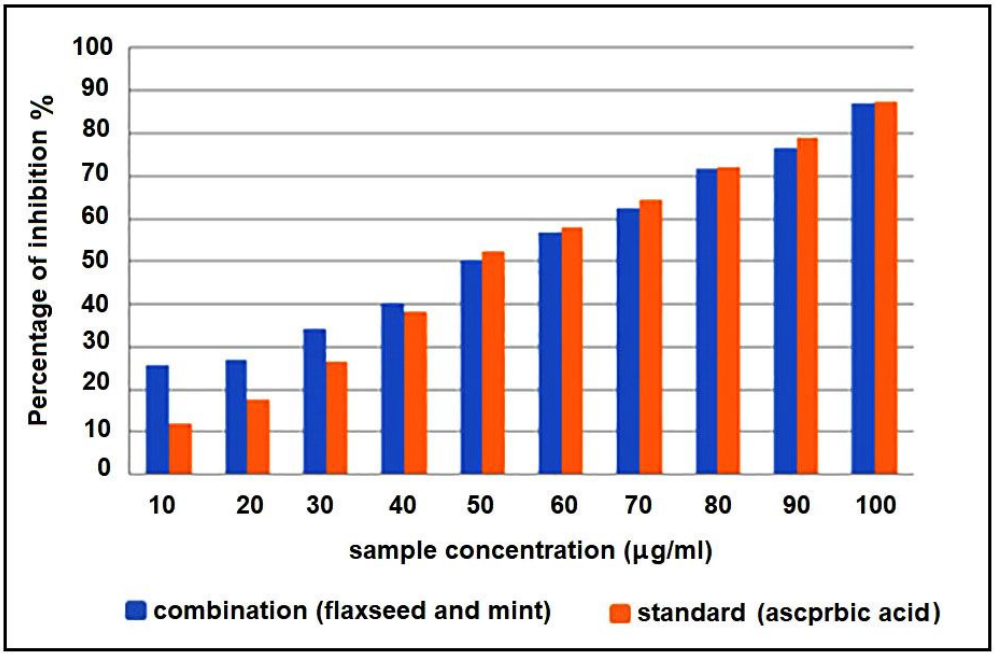

Fig 3: shows the percentage of inhibition of combination (*Linum usitatissimum* and *Mentha spicata*) extract at different concentrations and standard ascorbic acid using ABTS assay.

Table 5: *Linum usitatissimum*- Sample Concentration and Their Corresponding Percentage of Inhibition at Varying Concentration Using ABTS Assay

Sample concentration (µ g/ml)	10	20	30	40	50	60	70	80	90	100
% of inhibition	8.3	13.6	26.9	31.9	35.9	42.1	50.2	54.3	60.3	68.6

Table 6: *Mentha Spicata*- Sample Concentration and Their Corresponding Percentage of Inhibition at Varying Concentration using ABTS Assay

Sample concentration (µ g/ml)	10	20	30	40	50	60	70	80	90	100
% of inhibition	17.8	23.1	33.3	38.8	49.2	54.1	61.7	68	74.6	82

Table 7: Standard (Ascorbic Acid)- Sample Concentration and Their Corresponding Percentage of Inhibition at Varying Concentrationusing ABTS Assay

Sample concentration (µ g/ml)	10	20	30	40	50	60	70	80	90	100
% of inhibition	12	17.5	26.6	38.2	52.2	57.8	64.3	72.3	78.9	87.4

Table 8: Combination (*Linum usitatissimum* and *Mentha Spicata*)- Sample Concentration and Their Corresponding Percentage of Inhibition at Varying Concentrationusing ABTS Assay

Sample concentration (µg/ml)	10	20	30	40	50	60	70	80	90	100
% of inhibition	25.6	26.9	34.3	40.4	50.5	56.8	62.4	71.9	76.4	87

In our study, by ABTS assay, at a maximum concentration of 100µg/ml, the percentage of inhibition produced by *Linum usitatissimum*, *Mentha spicata*, and the combination is 68.6%,82%,87% respectively as depicted in figure 4. By comparing, we conclude combining the two extracts possesses better antioxidant activity than individual extracts, thus showing synergism.

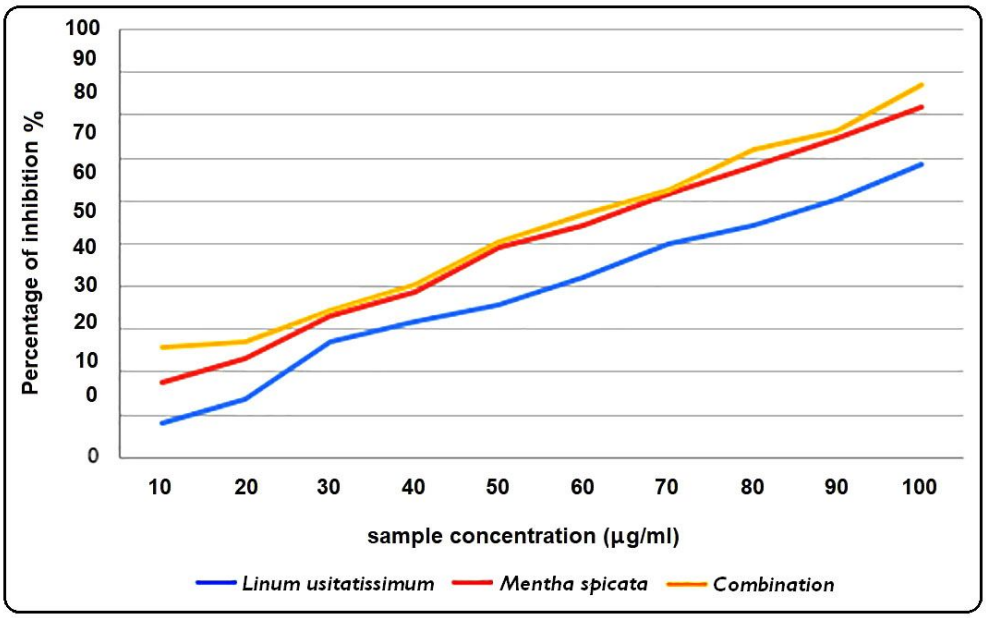

Fig 4: shows the percentage of inhibition of extracts of *Linum usitatissimum*, *Mentha spicata*, and the combination at different concentrations by ABTS assay.

Ferric-reducing antioxidant activity of *Linum usitatissimum* and *Mentha spicata* was assayed. According to the study by Waszkowiak et al., the ethanolic extract's reducing activity was about 7–9 times higher than the activity of aqueous extract obtained from the same flaxseed variety.[53] In our study, the reducing power of *Linum usitatissimum* increased in a concentration-dependent manner, as depicted in Table 9. It showed that the maximum reducing power was obtained at a 500µg/ml concentration. The reducing power of *Mentha spicata* at varying concentrations in our study is depicted in Table 10. All extracts can reduce Fe^{3+} to Fe^{2+} since increasing the concentration increases the absorbance of the extracts measured at 593 nm. The effective concentration (EC_{50}) was 70µg/mL. According to Abdelbasset et al., *Mentha spicata* extract exhibited an effective concentration (EC_{50}) of 289.5µg/mL in the concentration range studied.[54] The reducing power of the combination of *Linum usitatissimum* and *Mentha spicata* at varying concentrations was assessed and depicted in Table 11. It shows that the combination possesses superior antioxidant properties compared to the individual extracts. Hydrogen peroxide scavenging was assessed by the Fox reagent method. *Linum usitatissimum* showed the maximum scavenging of 64.5% at 500µg/ml, and IC_{50} was 345µg/ml. (Table 12). According to the study by Chera et al., IC_{50} of ethanolic extract of *Linum usitatissimum* was found to be 14.33.[40] *Mentha spicata* showed the maximum scavenging of 66.3% at 500µg/ml, and IC_{50} was found to be 330 µg/ml. (Figure 10, Table 13). A study done by Aldoghachi et al. showed maximum scavenging of 87.83% at 100µg/ml with IC_{50} of 28.12 by H_2O_2 assay.[55] Combination of *Linum usitatissimum* and *Mentha spicata* showed maximum scavenging of 68.7% at 500µg/ml compared to the standard, which showed 80% at 100 µg/ml. IC_{50} of the combination was found to be 300 µg/ml. (Figure 5, Table 14, and Table 15).

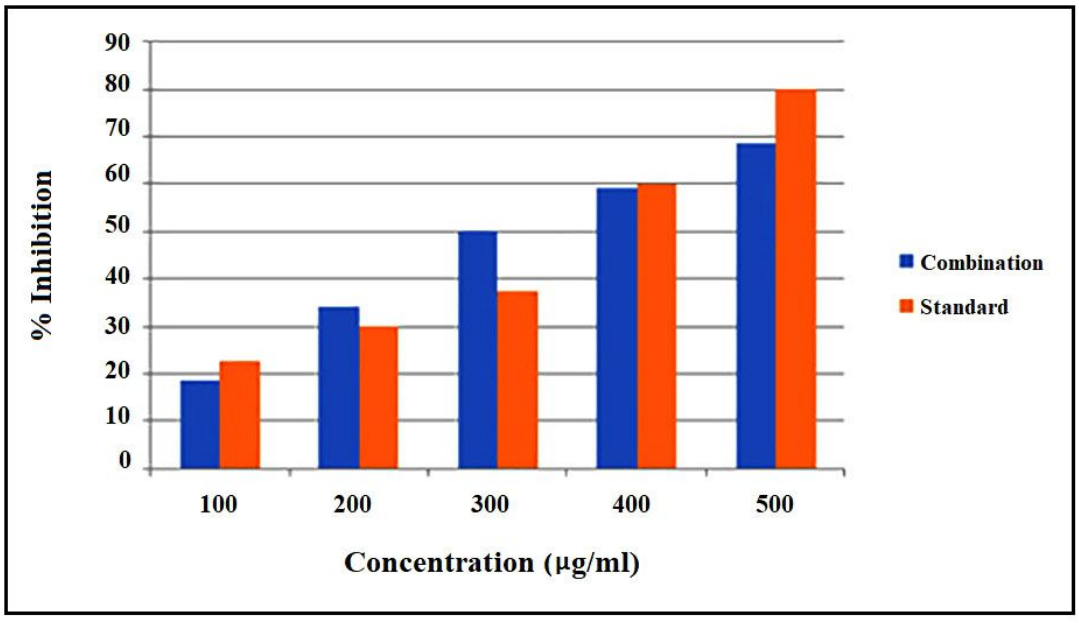

Fig 5: shows the percentage of inhibition of a combination of extracts of *Linum usitatissimum* and *Mentha spicata* compared with the standard (ascorbic acid) at different concentrations by H_2O_2 assay.

Table 9: *Linum usitatissimum* Sample Concentration and Their Corresponding Reducing Power at Varying Concentrations Using FRAP Assay					
Concentration (µg/ml)	**100**	**200**	**300**	**400**	**500**
Reducing power (µg)	50	70	80	100	130

Table 10: *Mentha Spicata* Sample Concentration and Their Corresponding Reducing Power at Varying Concentrations Using FRAP Assay					
Concentration (µg/ml)	**100**	**200**	**300**	**400**	**500**
Reducing power(µg)	60	70	90	110	120

Table 11: Combination (*Linum usitatissimum* and *Mentha Spicata*) Sample Concentration and Their Corresponding Reducing Power at Varying Concentrations Using FRAP Assay					
Concentration (µg/ml)	**100**	**200**	**300**	**400**	**500**
Reducing power(µg)	60	80	90	110	130

Table 12: *Linum usitatissimum* Concentration and Their Corresponding Percentage of Inhibition at Varying Concentration Using H_2O_2 Assay					
Concentration (µg/ml)	**100**	**200**	**300**	**400**	**500**
% inhibition	16.35	32.8	47	54	64.5

Table 13: *Mentha Spicata* Concentration and Their Corresponding Percentage of Inhibition at Varying Concentration Using H_2O_2 Assay					
Concentration (µg/ml)	**100**	**200**	**300**	**400**	**500**
% inhibition	15	33.2	48.8	57.9	66.3

Table 14: Standard (Ascorbic Acid) Concentration and Their Corresponding Percentage of Inhibition at Varying Concentration Using H_2O_2 Assay					
Concentration (µg/ml)	**20**	**40**	**60**	**80**	**100**
% inhibition	22.5	30.0	37.5	60.0	80.0

Table 15: Combination (*Linum usitatissimum* and *Mentha Spicata*) Concentration and Their Corresponding Percentage of Inhibition at Varying Concentration Using H_2O_2 Assay					
Concentration (µg/ml)	**100**	**200**	**300**	**400**	**500**
%inhibition	18.75	34.3	50	59.3	68.7

In our study, by H_2O_2 assay, at the maximum concentration of 500µg/ml, the percentage of inhibition produced by *Linum usitatissimum*, *Mentha spicata*, and the combination were 64.5%, 66.3%, and 68.7%, respectively as depicted in figure 6. It showed that *Linum usitatissimum* and *Mentha spicata* are good scavengers of H_2O_2. We conclude that the combination offers better synergistic antioxidant potential than individual extracts.

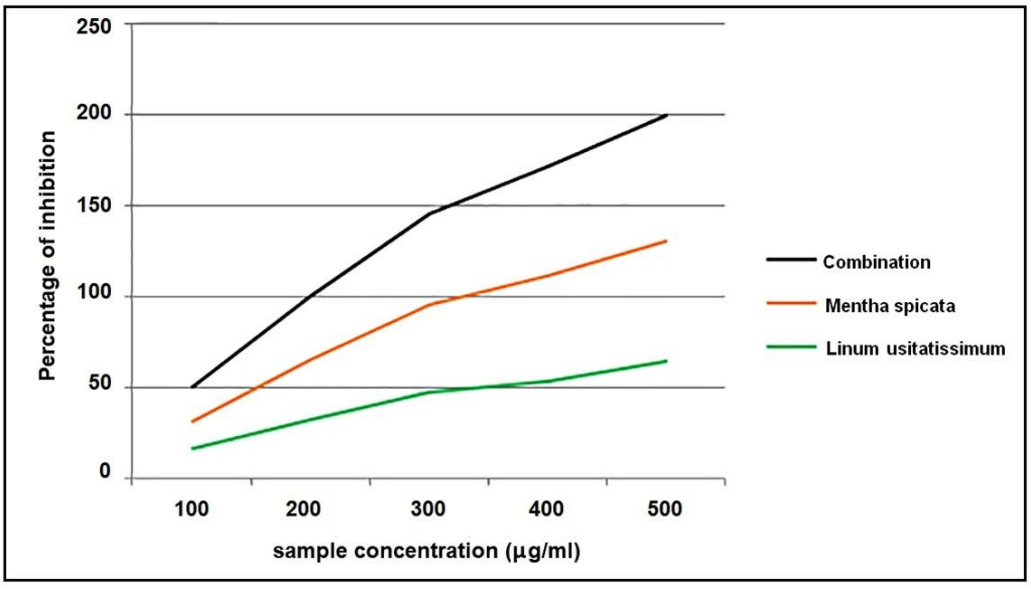

Fig 6: shows the percentage of inhibition of the combination of extracts of *Linum usitatissimum*, *Mentha spicata*, and the combination at different concentrations by H_2O_2 assay.

Hydroxyl Radical Scavenging Activity of *Linum usitatissimum* and *Mentha spicata* was calculated, which was measured spectrophotometrically at 532 nm. *Linum usitatissimum* showed the maximum scavenging of 72.8% at a concentration of 500µg/ml, and IC_{50} was found to be 185µg/ml. (Table 16). According to the study by Marambe et al., IC_{50} was 300µg/ml.[56] *Mentha spicata* showed maximum scavenging of 81.2% at a concentration of 500µg/ml, and IC50 was 130µg/ml. (Table 17). According to the study done by Dorman et al., IC50 was found to be 300µg/ml.[57] *Linum usitatissimum* and *Mentha spicata* combination showed the maximum scavenging of 87.8% at 500µg/ml compared to the standard, which showed 75.3% at 100 µg/ml. Furthermore, the IC50 of the combination was found to be 97µg/ml. (Figure 7, Table 18, and Table 19). It showed that the combination offers superior antioxidant activity than the standard (ascorbic acid).

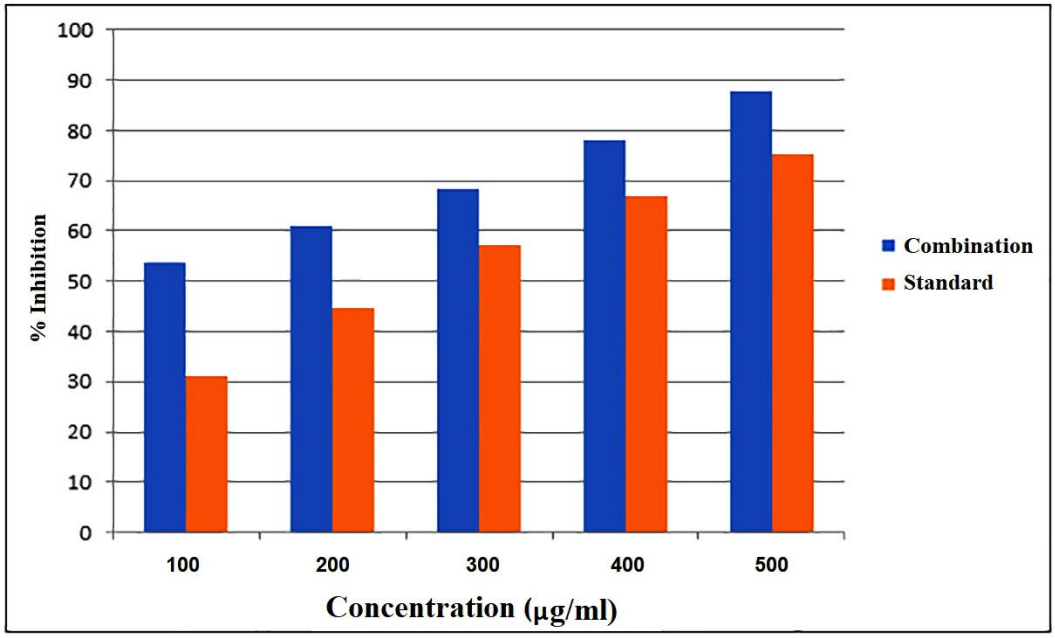

Fig 7: shows the percentage of inhibition of a combination of extracts of *Linum usitatissimum* and *Mentha spicata* compared with the standard (ascorbic acid) by hydroxyl radical scavenging assay.

Table 16: *Linum usitatissimum* Concentration and Their Corresponding Percentage of Inhibition at Varying Concentration Using Hydroxyl Radical Scavenging Assay

Concentration(µg/ml)	100	200	300	400	500
%inhibition	40.3	52.4	57.1	66	72.8

Table 17: *Mentha Spicata* Concentration and Their Corresponding Percentage of Inhibition at Varying Concentration Using Hydroxyl Radical Scavenging Assay

Concentration(µg/ml)	100	200	300	400	500
%inhibition	48	58.8	63.5	75.1	81.2

Table 18: Standard (Ascorbic Acid) Concentration and Their Corresponding Percentage of Inhibition at Varying Concentration Using Hydroxyl Radical Scavenging Assay

Concentration(µg/ml)	100	200	300	400	500
%inhibition	31.1	44.8	57.2	66.8	75.3

Table 19: Combination (*Linum usitatissimum* and *Mentha Spicata*) Concentration and Their Corresponding Percentage of Inhibition at Varying Concentration Using Hydroxyl Radical Scavenging Assay

Concentration (µg)	100	200	300	400	500
% inhibition	53.6	60.9	68.2	78	87.8

In our study, by hydroxyl radical scavenging assay, at the maximum concentration of 500µg/ml, the percentages of inhibition produced by *Linum usitatissimum*, *Mentha spicata*, and the combination were 72.8%, 81.2%, and 87.8% respectively as depicted in figure 8. It implies that the combination offers enhanced antioxidant potential than the standalone extracts. Synergism implies that when two or more herbal ingredients are combined, they mutually augment each other's effect more than the simple summation of these ingredients. As the definition declares, our study has proved that the combination exhibits a better antioxidant effect than the sum of the effect of individual compounds.

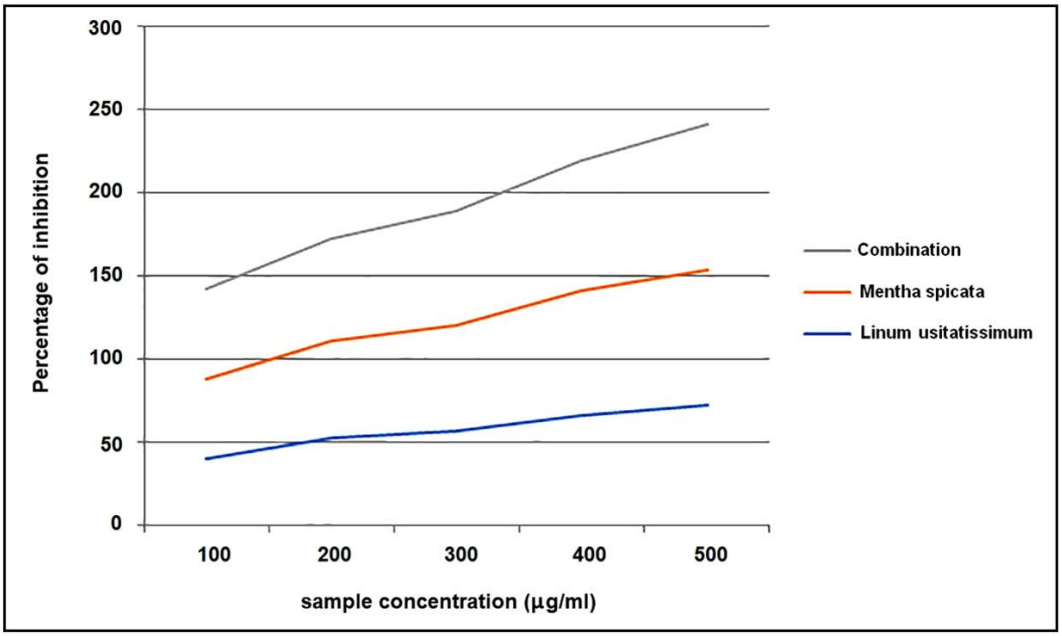

Fig 8: Synergistic anti-oxidant effect of *Linum usitatissimum* and *mentha spicata* extracts using hydroxyl radical scavenging assay.

4. CONCLUSION

Our study proved that combining flaxseed extract (*Linum usitatissimum*) and mint leaf extract (*Mentha spicata*) has a synergistic *in vitro* anti-oxidant effect. Also, the gastric side effects of *Linum usitatissimum*can be combated when combined with *Mentha spicata.* However, further animal studies and clinical trials are required to confirm the therapeutic application of this combination (*Linum usitatissimum* and *Mentha spicata*).

5. AUTHORS CONTRIBUTION STATEMENT

Ashwini T contributed to the conception, study design, and data analysis. Arul Amutha Elizabeth contributed to the study process and data acquisition. Ramya Ravichandar contributed to the literature search and drafting of the manuscript. All the authors read and approved the final manuscript.

6. ABBREVIATIONS

ORAC-Oxygen Radical Absorption Capacity
HORAC-Hydroxyl Radical Antioxidant Capacity
TRAP-Total Peroxyl Radical Trapping Antioxidant Parameter
CUPRAC-Cupric Reducing Antioxidant Power
FRAP-Ferric Reducing Antioxidant Power
ABTS-2,20 -Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid
DPPH-[2,2-di(4-tert-octyl phenyl)- 1-picrylhydrazyl]

7. CONFLICT OF INTEREST

Conflict of interest declared none.

8. REFERENCES

1. Phaniendra A, Jestadi DB, Periyasamy L. Free radicals: properties, sources, targets, and implications in various diseases. Indian J Clin Biochem. 2015;30(1):11-26. doi: 10.1007/s12291-014-0446-0, PMID 25646037.
2. Sen S, Chakraborty R. The role of antioxidants in human health. In: Oxidative stress: diagnostics, prevention, and therapy; 2011. p. 1-37. doi: 10.1021/bk-2011-1083.ch001.
3. Bhatti JS, Bhatti GK, Reddy PH. Mitochondrial dysfunction and oxidative stress in metabolic disorders - A step towards mitochondria based therapeutic strategies. Biochim Biophys Acta Mol Basis Dis. 2017;1863(5):1066-77. doi: 10.1016/j.bbadis.2016.11.010, PMID 27836629.
4. Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, et al. Oxidative stress: harms and benefits for human health. Oxid Med Cell Longev. 2017;2017:8416763. doi: 10.1155/2017/8416763, PMID 28819546.
5. Calleja C, Barros L, Antonio AL, Ćirić A, Soković M, Oliveira MBPP, et al. *Foeniculum vulgare Mill.* as natural conservation enhancer and health promoter by incorporation in cottage cheese. J Funct Foods. 2015;12:428-38. doi: 10.1016/j.jff.2014.12.016.
6. Iqbal T, Hussain AI, Chatha SA, Naqvi SA, Bokhari TH. Antioxidant activity and volatile and phenolic profiles of essential oil and different extracts of wild mint (*Mentha longifolia*) from the Pakistani Flora. J Anal Methods Chem. 2013;2013:536490. doi: 10.1155/2013/536490, PMID 24224115.
7. Furuta S, Nishiba Y, Suda I. Fluorometric assay for screening antioxidative activities of vegetables. J Food Sci. 1997;62(3):526-8. doi: 10.1111/j.1365-2621.1997.tb04422.x.
8. Wang H, Cao G, Prior RL. Total antioxidant capacity of fruits. J Agric Food Chem. 1996;44(3):701-5. doi: 10.1021/jf950579y.
9. Lin JK, Lin CL, Liang YC, Lin-Shiau SY, Juan IM. Survey of catechins, gallic acid, and methylxanthines in green, oolong, pu-erh, and black teas. J Agric Food Chem. 1998;46(9):3635-42. doi: 10.1021/jf980223x.
10. Vieira JM, Mantovani RA, Raposo MFJ, Coimbra MA, Vicente AA, Cunha RL. Effect of extraction temperature on rheological behavior and antioxidant capacity of flaxseed gum. Carbohydr Polym.

2019;213:217-27. doi: 10.1016/j.carbpol.2019.02.078, PMID 30879663.
11. Bordón MG, Meriles SP, Ribotta PD, Martinez ML. Enhancement of composition and oxidative stability of chia (*Salvia hispanica L.*) Seed oil by blending with specialty oils. J Food Sci. 2019;84(5):1035-44. doi: 10.1111/1750-3841.14580, PMID 30990893.
12. Yeasmin A, Junaid Md, Syeda A, Afsana N, Muhammad A, Tania S, et al. A comprehensive review on *Linum usitatissimum* medicinal plant: its phytochemistry, pharmacology, and ethnomedicinal uses. Mini Rev Med Chem. 2021;21(18).
13. Padmini E, Prema K, Vijaya Geetha B, Usha Rani M. Comparative study on composition and antioxidant properties of mint and black tea extract. Int J Food Sci Technol. 2008;43(10):1887-95. doi: 10.1111/j.1365-2621.2008.01782.x.
14. Deep A, Rana P, Gosal S, Soni G. Antioxidant potential of tissue cultured *Mentha spicata*. Biomed Rx. 2013;1(1):90-6.
15. Mahboubi M. *Mentha spicata L.* essential oil, phytochemistry and its effectiveness in flatulence. J Trad Complement Med. 2021;11(2):75-81. doi: 10.1016/j.jtcme.2017.08.011, PMID 33728265.
16. Gholaminejhad M, Arabzadeh S, Akbari M, Mohamadi Y, Hassanzadeh G. Anti-oxidative and neuroprotective effects of flaxseed on experimental unilateral spinal cord injury in the rat. J Contemp Med Sci. 2017;3(10):213-7. doi: 10.22317/jcms.06201703.
17. Rajesha J, Murthy KNC, Kumar MK, Madhusudhan B, Ravishankar GA. Antioxidant potentials of flaxseed by in vivo model. J Agric Food Chem. 2006;54(11):3794-9. doi: 10.1021/jf053048a, PMID 16719498.
18. Zeb A. Concept, mechanism, and applications of phenolic antioxidants in foods. J Food Biochem. 2020;44(9):e13394. doi: 10.1111/jfbc.13394, PMID 32691460.
19. Anwar F, Przybylski R. Effect of solvent extraction on total phenolics and antioxidant activity of extracts from flaxseed (*Linum usitatissimum L.*). Acta Sci Pol Technol Aliment. 2012;11(3):293-301. PMID 22744950.
20. Fletcher RS, Slimmon T, Kott LS. Environmental factors affect rosmarinic acid accumulation in spearmint (L.) and peppermint (L.). Open Agric J. 2010;4(1):10-6. doi: 10.2174/1874331501004010010.
21. Sierra K, Naranjo L, Carrillo-Hormaza L, Franco G, Osorio E. Spearmint (*Mentha spicata L.*) phytochemical profile: impact of pre/post-harvest processing and extractive recovery. Molecules. 2022;27(7):2243. doi: 10.3390/molecules27072243, PMID 35408640.
22. Malik B, Sharma NR, Soni G. Influence of agro-climatic conditions on the anti-oxidant potential of *Mentha* species. J Pharm Res. 2013;7(5):427-32. doi: 10.1016/j.jopr.2013.05.014.
23. Williamson EM. Synergy and other interactions in phytomedicines. Phytomedicine. 2001;8(5):401-9. doi: 10.1078/0944-7113-00060, PMID 11695885.
24. Kristensen M, Jensen MG, Aarestrup J, Petersen KE, Søndergaard L, Mikkelsen MS, et al. Flaxseed dietary fibers lower cholesterol and increase fecal fat excretion. Still, the magnitude of the effect depends on the food type. Nutr Metab. 2012;9(1):1-8.
25. Mehraban M, Jelodar G, Rahmanifar F. A combination of spearmint and flaxseed extract improved endocrine and histomorphology of ovary in experimental PCOS. J Ovarian Res. 2020;13(1):32. doi: 10.1186/s13048-020-00633-8, PMID 32197626.
26. Alam MN, Bristi NJ, Rafiquzzaman M. Review on in vivo and in vitro methods evaluation of the antioxidant activity. Saudi Pharm J. 2013;21(2):143-52. doi: 10.1016/j.jsps.2012.05.002, PMID 24936134.
27. Oraemesi I. Soxhlet extraction of avocado endocarp and trituration of avocado mesocarp for biodiesel production; 2017. Master's theses. 13. Available from: https://scholars.fhsu.edu/theses/13/.
28. Zduńska K, Dana A, Kolodziejczak A, Rotsztejn H. Antioxidant properties of ferulic acid and its possible application. Skin Pharmacol Physiol. 2018;31(6):332-6. doi: 10.1159/000491755, PMID 30235459.
29. Koleva II, van Beek TA, Linssen JP, de Groot A, Evstatieva LN. Screening plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochem Anal. 2002;13(1):8-17. doi: 10.1002/pca.611, PMID 11899609.
30. Akram NA, Shafiq F, Ashraf M. Ascorbic acid-A potential oxidant scavenger and its role in plant development and abiotic stress tolerance. Front Plant Sci. 2017;8:613. doi: 10.3389/fpls.2017.00613, PMID 28491070.
31. Panigrahy SK, Kumar A, Bhatt R. Antioxidant potentials of successive solvent extracts from the new Hedhychiumcoronarium rhizome. J Food Sci Technol. 2017;54(10):3297-306. doi: 10.1007/s13197-017-2777-3, PMID 28974815.
32. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem. 1996;239(1):70-6. doi: 10.1006/abio.1996.0292, PMID 8660627.
33. Kowsalya R, Elangovan N. In vitro antioxidant and free radical scavenging activity of isolongifolene. Asian J Biol Sci. 2014;7:13-23.
34. Ramakrishna H, Murthy S, Murthy GP. Hydroxy radical and DPPH scavenging activity of crude protein extract of *Leucas linifolia*: A folk medicinal plant. Asian J Plant Sci Res. 2012;2.
35. Prasad K. Hydroxyl radical-scavenging property of secoisolariciresinol diglucoside (SDG) isolated from flax-seed. Mol Cell Biochem. 1997;168(1-2):117-23. doi: 10.1023/a:1006847310741, PMID 9062900.
36. Aykul S, Martinez-Hackert E. Determination of half-maximal inhibitory concentration using biosensor-based protein interaction analysis. Anal Biochem. 2016;508:97-103. doi: 10.1016/j.ab.2016.06.025, PMID 27365221.
37. Liang S, Liao W, Ma X, Li X, Wang Y. H_2O_2 oxidative preparation, characterization and antiradical activity of a novel oligosaccharide derived from flaxseed gum. Food Chem. 2017;230:135-44. https :. doi: 10.1016/j.foodc, PMID 28407893 hem.2017.03.029.
38. Deme T, Haki GD, Retta N, Woldegiorgis A, Geleta M. Fatty acid profile, total phenolic content, and antioxidant activity of Niger seed (*Guizotia abyssinica*) and linseed (*Linum usitatissimum*). Front Nutr. 2021;8:674882. doi: 10.3389/fnut.2021.674882, PMID 34409060.
39. Amin T, Thakur M. A comparative study on proximate composition, phytochemical screening, antioxidant and antimicrobial activities of *Linum usitatisimum L.* (flaxseeds). Int J Curr Microbiol Appl Sci. 2014;3:465-81.

40. Chera EI, Pop RM, Pârvu M, Soriţău O, Uifălean A, Cătoi FA, et al. Flaxseed ethanol extracts' antitumor, antioxidant, and anti-inflammatory potential. Antioxidants (Basel). 2022;11(5):892. doi: 10.3390/antiox11050892, PMID 35624757.
41. Han H, Yılmaz H, Gülçin İ. Antioxidant activity of flaxseed (*Linum usitatissimum L.*) Shell and analysis of its polyphenol contents by LC-MS/MS. Rec Nat Prod. 2018;12(4):397-402. doi: 10.25135/rnp.46.17.09.155.
42. Choudhury RP, Kumar A, Garg AN. Analysis of Indian mint (*Mentha spicata*) for essential, trace, and toxic elements and its antioxidant behavior. J Pharm Biomed Anal. 2006;41(3):825-32. doi: 10.1016/j.jpba.2006.01.048, PMID 16504451.
43. El Menyiy N, Mrabti HN, El Omari N, Bakili AE, Bakrim S, Mekkaoui M, et al. Medicinal uses, phytochemistry, pharmacology, and toxicology of *Mentha spicata*. Evid Based Complement Alternat Med. 2022;2022:7990508. doi: 10.1155/2022/7990508, PMID 35463088.
44. Dhifi W, Jelali N, Mnif W, Litaiem M, Hamdi N. Chemical composition of the essential oil of *Mentha spicata L.* from Tunisia and its biological activities. J Food Biochem. 2013;37(3):362-8. doi: 10.1111/j.1745-4514.2012.00656.x.
45. Selles SMA, Kouidri M, Bellik Y, Amrane AA, Belhamiti BT, Benia AR, et al. Chemical composition, antioxidant and in vitro antibacterial activities of *Mentha spicata* leaf essential oils from Tiaret area (Algeria). Dhaka Univ J Pharm Sci. 2018;17(1):87-96. doi: 10.3329/dujps.v17i1.37123.
46. Brahmi F, Adjaoud A, Marongiu B, Falconieri D, Yalaoui-Guellal D, Madani K, et al. Chemical and biological profiles of essential oils from *Mentha spicata L.* leaf from Bejaia in Algeria. J Essent Oil Res. 2016;28(3):211-20. doi: 10.1080/10412905.2015.1118411.
47. Safdar B, Pang Z, Liu X, Jatoi MA, Mehmood A, Rashid MT, et al. Flaxseed gum: extraction, bioactive composition, structural characterization, and its potential antioxidant activity. J Food Biochem. 2019;43(11):e13014. doi: 10.1111/jfbc.13014, PMID 31475377.
48. Liang S, Liao W, Ma X, Li X, Wang Y. H_2O_2 oxidative preparation, characterization and antiradical activity of a novel oligosaccharide derived from flaxseed gum. Food Chem. 2017;230:135-44. https :. doi: 10.1016/j.foodchem.2017.03.029, PMID 28407893.
49. Bouaziz F, Koubaa M, Barba FJ, Roohinejad S, Chaabouni SE. Antioxidant properties of water-soluble gum from flaxseed hulls. Antioxidants (Basel). 2016;5(3):26. https ::x5030026. doi: 10.3390/anti, PMID 27490574.
50. Fatiha B, Didier H, Naima G, Khodir M, Martin K, Léocadie K, et al. Phenolic composition, in vitro antioxidant effects and tyrosinase inhibitory activity of three Algerian Mentha species: *M. spicata* (*L.*)*, M. pulegium* (*L.*) *and M. rotundifolia* (*L.*) *Huds* (*Lamiaceae*). Ind Crops Prod. 2015;74:722-30. doi: 10.1016/j.indcrop.2015.04.038.
51. Nickavar B, Alinaghi A, Kamalinejad M. Evaluation of the antioxidant properties of five mentha species. Iran J Pharm Res. 2008;7(3):203-9.
52. Arumugam P, Ramamurthy P, Ramesh A. Antioxidant and cytotoxic activities of lipophilic and hydrophilic fractions of *Mentha spicata L.* (Lamiaceae). Int J Food Prop. 2010;13(1):23-31. doi: 10.1080/10942910802144329.
53. Waszkowiak K, Gliszczyńska-Świgło A, Barthet V, Skręty J. Effect of extraction method on the phenolic and cyanogenic glucoside profile of flaxseed extracts and their antioxidant capacity. J Am Oil Chem Soc. 2015;92(11-12):1609-19. doi: 10.1007/s11746-015-2729-x, PMID 26640278.
54. Kaddour A, Amara DG, Moussaoui Y, Chemsa AE, Alia Z, Kamarchou A. Total phenolic and flavonoid contents of *Mentha spicata* leaves aqueous extracts in different regions of Algeria and their antioxidant and antidiabetic activities. Trop J Pharm Res. 2022;21(9):1907-13. doi: 10.4314/tjpr.v21i9.14.
55. Aldoghachi FEH, Noor Al-Mousawi UM, Shari FH. Antioxidant activity of rosmarinic acid extracted and purified from *Mentha piperita*. Arch Razi Inst. 2021;76(5):1279-87. doi: 10.22092/ari.2021.356072.1770, PMID 35355734.
56. Marambe PWMLHK, Shand PJ, Wanasundara JPD. An in-vitro investigation of selected biological activities of hydrolyzed flaxseed (*Linum usitatissimum L.*) proteins. J Am Oil Chem Soc. 2008;85(12):1155-64. doi: 10.1007/s11746-008-1293-z.
57. Dorman HJ, Koşar M, Kahlos K, Holm Y, Hiltunen R. Antioxidant properties and composition of aqueous extracts from Mentha species, hybrids, varieties, and cultivars. J Agric Food Chem. 2003;51(16):4563-9. doi: 10.1021/jf034108k, PMID 14705878.