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INVESTIGATION OF ANTIOXIDANT POTENTIAL OF INDIVIDUAL AND COMBINATION OF *CYMBOPOGON CITRATUS*, *AZADIRACTA INDICA*, AND *CURCUMA LONGA* EXTRACTS

Manmohan Sharma[1*], Hariom Sharma[1], Girish Kumar Vyas[1], Anil Sharma[1], Gautam Kumar[2]

[1] School of Pharmaceutical Studies, Faculty of Health Sciences, Dr. K. N. Modi University, Newai-304021, Rajasthan.

[2] School of Pharmacy, Sharda University, Greater Noida-201310, Uttar Pradesh.

***Corresponding author**

Mr. Manmohan Sharma

Associate Professor,

School of Pharmaceutical Studies, Faculty of Health Sciences,

Dr. K. N. Modi University, Newai-304021, Rajasthan.

Tel: 9460718580

E-mail: sharma.manmohan30@gmail.com

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Abstract

Background: In the food and herbal medicine sector, substances like turmeric (*Curcuma longa*), neem leaves (*Azadirachta indica*), and lemongrass (*Cymbopogon citratus*) are widely employed. Recent reports indicated that these plants contain some phytochemicals which are known to have antioxidant properties. However, the synergistic antioxidant effects of the combination of these medicinal plant extracts have not been reported to date. This study was conducted to investigate the antioxidant potentials of individual and combination of turmeric, neem leaves, and lemongrass extracts. *Methodology:* The 2, 2-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging assay was used to assess the antioxidant activity of all the fresh plant extracts. There were 16 formulation trials with different proportions of extracts selected randomly. *Results:* When samples *viz*. LG 50%, NE 50%, and CE 50% extracts antioxidant activity are compared, it was found that Neem leaf extract showed the highest antioxidant activity followed by lemongrass leaves and turmeric extracts and the % antioxidant were recorded as 76.61%, 66.72% and 62.04% respectively. When samples containing LG 100%, NE 100% and CE 100% are compared, the result showed that the radical scavenging activity

of Neem leaf and lemon grass leaf extracts were higher i.e. 94.43% and 92.17% respectively whereas, Turmeric extract gave only 87.17% radical scavenging activity. When two or three plant combinations are compared with individual plant extracts, the results showed that the combination of different extracts has significant antioxidant activity, indicating the synergistic interaction of the combination of two and three plant extracts. During the study, it was observed that some combinations of plant extracts showed significant antioxidant activity and some combinations of extracts showed non-significant activity. *Conclusion:* In this research study most plant combinations showed synergistic interactions in the DPPH radical scavenging assay. We suggest using only combinations of synergistic plant extracts for antioxidant activity should be used, and plant extracts having antagonistic activity should be avoided.

Keywords: Antioxidant, Plant extracts, DPPH assay, Synergistic antioxidant activity.

1. INTRODUCTION

Being a highly reactive chemical, oxygen causes harm to living things by creating reactive oxygen species. Free radical production and chain reactions were responsible for the disorders linked to oxidative stress [1]. Despite their surprising toxicity and side effects, synthetic antioxidants appear to be more effective. Researchers are now more interested in looking into the antioxidant capabilities of natural resources like plants and herbs as a result of this circumstance. Natural antioxidants found in most plants and herbs have synergistic interactions and effects. However, because phytochemical interactions are not always positive, they can also have negative, positive, or neutral effects [2,3].

Synergistic interaction is described as a favourable interaction when two or more drugs are combined, and the resulting mixture demonstrates a stronger mechanism than the sum of the individual components. Researchers and scientists who study antioxidants, antimicrobials, antifungals and the creation of novel drugs all employ the concept of synergism [4]. Antagonism, on the other hand, is the result of combination. The outcome, however, demonstrates that neither good nor bad is considered neutral or additive in terms of the aggregate effect of the mixture. In the food sector, substances like turmeric (*Curcuma longa*), neem leaves (Azadirachta indica), and lemongrass (*Cymbopogon citratus*) are widely employed. The antioxidant activities of these plants were demonstrated by prior studies to contain various phytochemicals [5–9]. The evaluations, however, were rarely done in such combinations. In order to better understand antioxidant qualities and their interactions with one another, the current investigation was carried out.

2. MATERIALS AND METHODS

2.1. Plant Collection

Neem leaves (Azadirachta indica), and lemongrass (*Cymbopogon citratus*) were collected from Dr. K. N. Modi University, Newai, university campus. *Curcuma longa* was collected from nearby the farmhouse.

2.2. Plant Authentication

It was identified and confirmed by the Principal of, the School of pharmaceutical studies, Faculty of Health Sciences of Dr. K. N. Modi University and HOD of the School of Agriculture Science of Dr. K. N. Modi University, Newai, Rajasthan. After these confirmations, it was authenticated by the Botany Department of Rajasthan University, Jaipur. The plant authentication no is RUBL21216 for Neem, RUBL21218 for lemongrass (*Cymbopogon citratus*) and RUBL21217 for *Curcuma longa*.

2.3. Procedure for extraction

Freshly collected dried plants Neem and lemongrass leaf materials and turmeric rhizome were used in the extraction of all ingredients. All powdered material was placed in a thimble and separately placed in a Soxhlet chamber. The required amount of 90% alcohol (ethanol) as a solvent was placed in a round bottom flask and assembled for a Soxhlet extractor, and then the distillation process was started. After the extraction process was completed, the extracts were placed open in a hot water bath to evaporate the solvent and obtain the dried extract.

2.4. Preparation of the sample

Before the examination, 250 mg of each extracted sample was accurately weighed and dissolved in 25 mL of 50% alcohol in separate tubes and covered with aluminium foil [12]. Different formulations were created randomly. In this study, 16 different plant extract formulations were examined. The preparation of the corresponding samples is shown in Table 1.

Table 1 shows the sample preparation layout.

Sample No.	Lemon Grass Extract (ml)	Neem Leaves extract (ml)	Turmeric extract (ml)	Distilled water (ml)
1	5	0	0	5
2	10	0	0	0
3	5	5	0	0
4	5	0	5	0
5	0	5	0	5

6	0	10	0	0
7	5	5	0	0
8	0	5	5	0
9	0	0	5	5
10	0	0	10	0
11	5	0	5	0
12	0	5	5	0
13	7	1.5	1.5	0
14	1.5	7	1.5	0
15	1.5	1.5	7	0
16	3.33	3.33	3.33	0

2.5. Radical scavenging activity

With a little adjustment by Miliaukas, the radical scavenging activity was carried out following the earlier Bondet approach [13, 14]. In 100 ml of methanol, 5.9 mg of 2,2-diphenyl-2-picryl hydrazyl (DPPH) solution was dissolved. To reduce the rate of oxidation, the solution was made every day. 77 ul of the sample was combined with three (3) ml of the DPPH solution. The samples were then maintained at room temperature for 15 minutes in the dark. The absorption at 515 nm was measured with a UV- Vis spectrophotometer. Three times the procedure was performed. The formula was then used to determine the radical scavenging activity (equation 1):

$$\% \text{ Inhibition} = \frac{(AA - AB)}{AA} X \quad 100 \ldots\ldots \text{Eq.1}$$

Where:

AB – absorption of the blank sample (t = 0min);

AA- absorption of tested extract solution (t = 15min)

2.6. Interaction effect

Hugo et al [15].'s calculations for the plant's interaction effects were somewhat modified. By adding the percentage of each reaction of each plant extract following the proportion of the mixtures, the projected values of the response were computed. In order to ascertain the interaction effects, the anticipated inhibition was compared to the experimental percentage [16].

2.7. Statistical analysis

The data were examined using an ordinary one-way analysis of variance (ANOVA) multiple comparison test followed by Tukey's posthoc test using GraphPad Prizm version 8.0.2 (comprehensive system for analyzing data powered by IBM), the statistically significant difference was then assessed at a minimum value of $p < 0.05$.

3. RESULT AND DISCUSSION

3.1. DPPH radical scavenging activity of single plant extracts

Each plant extract results are shown in Table 2. Based on the experiment, when sample 1 containing LG 50%, Sample 5 containing NE 50% and sample 9 containing CE 50% extracts antioxidant activity were compared, it was observed that Neem leaf extract showed the highest scavenging activity followed by lemongrass leaves and turmeric extracts and the values were 76.61±1.89%, 66.72±0.66% and 62.04±1.06% respectively. The antioxidant activity of Neem leaf and lemon grass leaf extracts was higher compared to turmeric extracts.

When sample 2 containing LG 100%, sample 6 containing NE 100% and sample 10 containing CE 100% are compared and the result of the radical scavenging activity of Neem leaf and lemon grass leaf extracts were recorded as 94.43±0.77% and 92.17±1.73% respectively whereas, Turmeric extract gave only 87.17±2.04% radical scavenging inhibition (Fig. 1). When sample containing 100% extracts are compared with sample containing 50 % extracts result show the increased antioxidant activity of sample containing 100% extracts.

Table 2: DPPH radical scavenging activity of single plant extracts according to standard run number.

Sample No.	% DPPH Scavenging activity
1	66.72±0.66
5	76.61±1.89
9	62.04±1.06
2	92.17±1.73
6	94.43±0.77
10	87.17±2.04

Note: Values are mean ± standard deviation (n = 3). Values with the same superscript letter within each column are not significantly different (p > 0.05).

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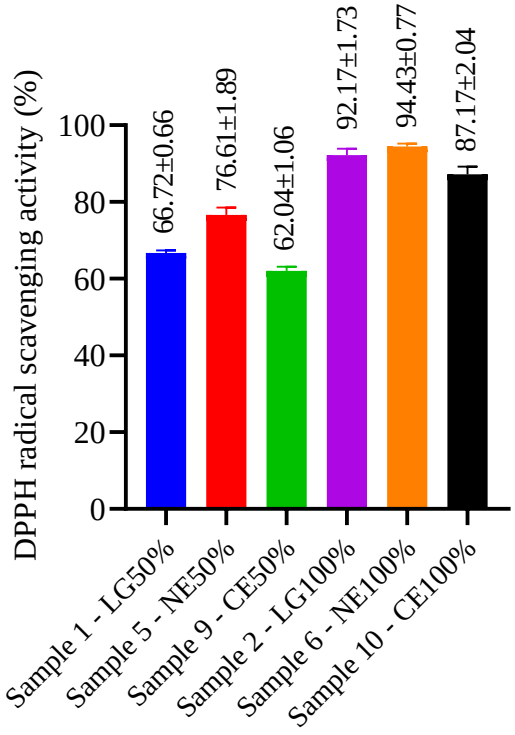

Figure 1: DPPH radical scavenging activity of lemongrass (LG), Neem leaves Extract (NE), and Curcuma extract (CE).

Note: LG – lemon grass extract; NE – neem leaves extract; CE – curcuma extract.

3.2. DPPH radical scavenging activity of combined plant extracts.

3.2.1. DPPH radical scavenging activity of individual Lemon grass extract and in combination with Neem leaves extract, and Curcuma extract.

Based on the experiment, the DPPH radical scavenging activity of Individual lemon grass extract (LG 50% and LG 100%) and in combination with Neem and Curcuma extract with different concentrations are compared as shown in Table 3 and Figure 2.

In this study samples 2, 3,4,13,14,15 and 16 are compared with sample 1 containing 50% LG extract and results showed that the combination of different extracts has significant antioxidant activity, indicating the synergistic interaction of the combination two and three plant extracts [17,18].

When samples 3,4,13,14,15 and 16 are compared with sample 2 containing 100% LG extract, the results were observed as followed- a) Sample 3 containing LG 50% +NE 50% showed increased antioxidant activity compared to sample 2 but not significant whereas, sample 4 containing LG 50% + CE 50% showed significant less antioxidant compared to sample 2. b) Sample 13 containing LG 70% +NE 15% +CE 15%, sample 14 containing LG 15% +NE 70% +CE 15% and sample 16 containing LG 33.3% + NE 33.3% + CE 33.3% showed nonsignificant antioxidant activity however, sample 15

containing LG 15% + NE 33.3% + CE 75% showed significant greater antioxidant activity compared to sample 2.

Table 3: DPPH radical scavenging activity of individual Lemon grass extract and in combination with Neem leaves extract, and Curcuma extract according to standard run number.

Sample No.	% DPPH Scavenging activity
1	66.72±0.66
2	92.17±1.73
3	95.37±0.74
4	78.34±1.47
13	92.64±1.74
14	94.93±0.93
15	87.89±1.79
16	90.50±1.26

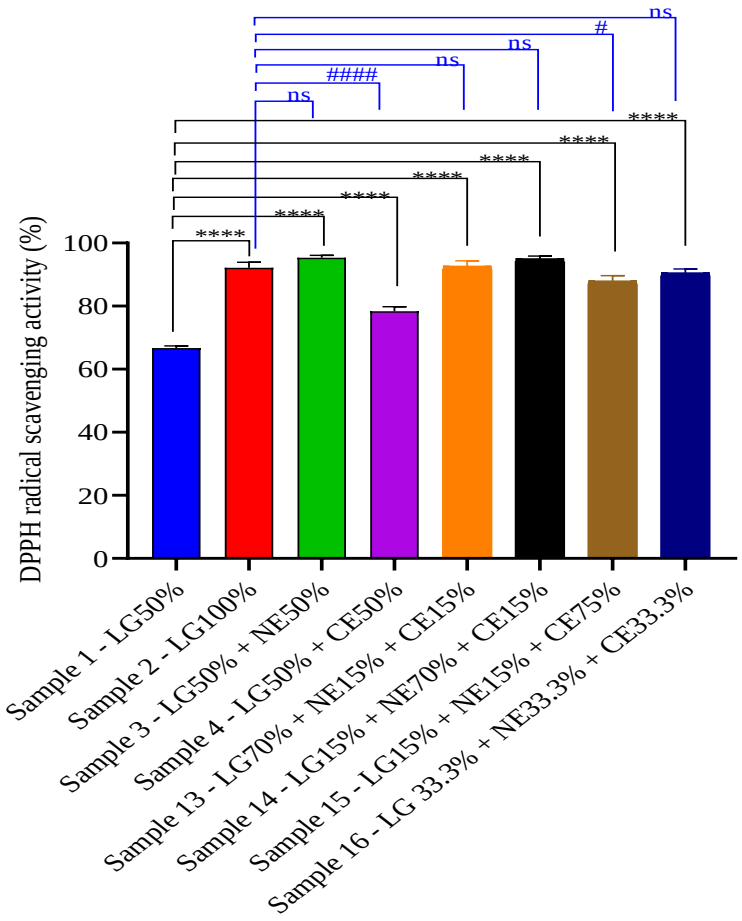

Figure 2: DPPH radical scavenging activity of individual Lemon grass extract and in combination with Neem leaves extract, and Curcuma extract.

Note: LG – lemon grass extract; NE – neem leaves extract; CE – curcuma extract. **** - $p<0.0001$; *** - $p<0.001$; ** - $p<0.01$; * - $p<0.05$; ns – non-significant. # - $p<0.05$, ### - $p<0.001$ Indicates increased antioxidant activity, # indicates decreased antioxidant activity.

3.2.2. DPPH radical scavenging activity of individual Neem leaves extract and in combination with Lemon grass extract, and Curcuma extract.

Based on the experiment, the DPPH radical scavenging activity of Individual neem extract (NE 50% and NE 100%) and in combination with Lemon Grass and Curcuma extract with different concentrations are compared as shown in Table 4 and Figure 3.

In this study samples 6, 7, 8, 13, 14, 15 and 16 are compared with sample 5 containing 50% NE extract and results showed that the combination of different extracts has significant antioxidant activity, indicating the synergistic interaction of the combination two and three plant extracts [17,18].

When sample 7 contains NE50%+LG 50%, sample 8 contains NE 50% +CE 50%, Sample 13 contains LG 70% +NE 15% +CE 15%, sample 14 contains LG 15 % +NE 70 % +CE 15%, sample 15 contains LG 15% +NE 15% +CE 75% and sample 16 LG 33.3% + NE 33.3%+ CE 33.3% compared with sample 6 containing 100% NE, the results were observed as followed- a) Sample 7 containing NE50%+LG 50% showed increased antioxidant activity compared to sample 6 containing 100% NE but not significant whereas sample 8 containing NE 50% +CE 50% showed significant less antioxidant compared to sample 6. b) Sample 13 containing LG 70% +NE 15% +CE 15% showed non-significant less antioxidant activity as compared to sample 6. Sample 14 containing LG 15 % +NE 70 % +CE 15% showed increased antioxidant activity but was not significant. c) Sample 15 containing LG 15% +NE 15% +CE 75% and sample 16 LG 33.3% + NE 33.3%+ CE 33.3% showed less antioxidant activity compared to sample 6. Sample 15 showed greater antioxidants compared to sample 16.

Table 4: DPPH radical scavenging activity of individual Neem leaves extract and in combination with Lemon grass extract, and Curcuma extract according to standard run number.

Sample No.	% DPPH Scavenging activity
5	76.61±1.89
6	94.43±0.78
7	95.37±0.74
8	85.58±1.15
13	92.64±1.74
14	94.93±0.93
15	87.89±1.79

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16	90.50±1.26

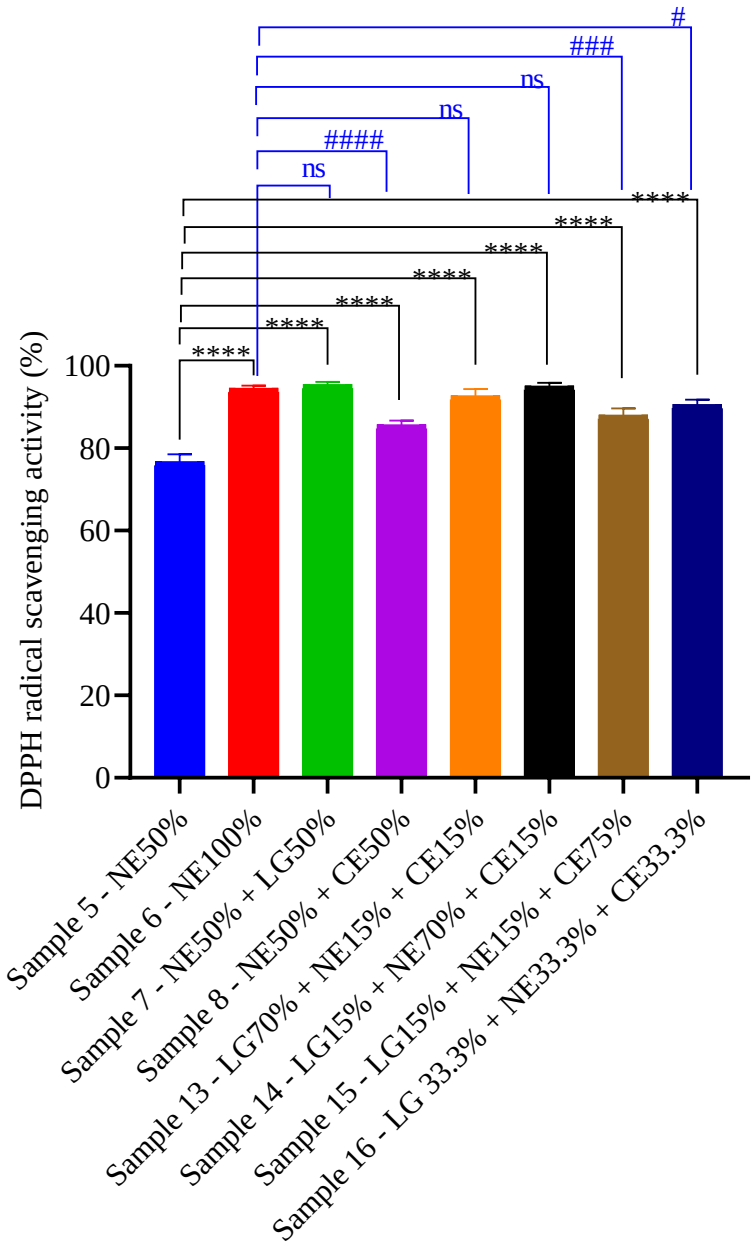

Figure 3: DPPH radical scavenging activity of individual Neem leaves extract and in combination with Lemon grass extract and Curcuma extract.

Note: NE – neem leaves extract; LG – lemon grass extract; CE – curcuma extract. **** - $p<0.0001$; *** - $p<0.001$; ** - $p<0.01$; * - $p<0.05$; ns – non-significant.

3.2.3. DPPH radical scavenging activity of individual Curcuma extract and in combination with Lemon grass extract, and Neem leaves extract.

Based on the experiment, the DPPH radical scavenging activity of Individual Curcuma extract (CE 50% and CE 100%) and in combination with Neem and Lemon Grass extract with different concentrations are compared as shown in Table 5 and Figure 4.

In this study sample 10 contains 100% CE, sample 11 contains CE 50%+LG50%, Sample 12 contains CE50 % +NE 50%, sample 13 contains LG 70 % +NE 15 % +CE 15%, sample 14containing LG 15 % +NE 70 % +CE 15%, sample 15 containing LG 15% +NE 15% +CE 75% and sample 16 containing 33.3%+NE 33.3%+CE33.3 % compared with sample 9 containing 50% CE and results showed that the combination of different extracts have significant antioxidant activity, indicating the synergistic interaction of the combination two and three plant extracts [19-21].

2.3.2 When sample 11containing CE 50%+LG50%, Sample 12 contains CE50 % +NE 50%, sample 13 containing LG 70 % +NE 15 % +CE 15%, sample 14containing LG 15 % +NE 70 % +CE 15%, sample 15 containing LG 15% +NE 15% +CE 75% and sample 16 containing 33.3%+NE 33.3%+CE33.3 % compared with sample 10 containing 100 % CE, the results were observed as followed- a) Sample 11 containing CE 50%+LG50% showed less antioxidant activity compared to sample 10 containing 100% CE but result is significant whereas 12 containing CE50 % +NE 50% showed non-significant less antioxidant activity. b) Sample 13 containing LG 70 % +NE 15 % +CE 15% and sample 14 containing LG 15 % +NE 70 % +CE 15% both showed increased antioxidant activity as compared to sample 10 containing CE 100% with significant results and sample 14 was more significant to sample 13. c) Sample 15 containing LG 15% +NE 15% +CE 75% and sample 16 containing 33.3%+NE 33.3%+CE33.3 % showed increased antioxidant activity compared to sample 10 however, the obtained results were non-significant.

Table 5: DPPH radical scavenging activity of individual Curcuma extract and in combination with Lemon grass extract, and Neem leaves extract according to standard run number.

Sample No.	% DPPH Scavenging activity
9	62.04±1.07
10	87.17±2.05
11	78.34±1.47
12	85.58±1.15
13	92.64±1.74
14	94.93±0.93
15	87.89±1.79
16	90.50±1.26

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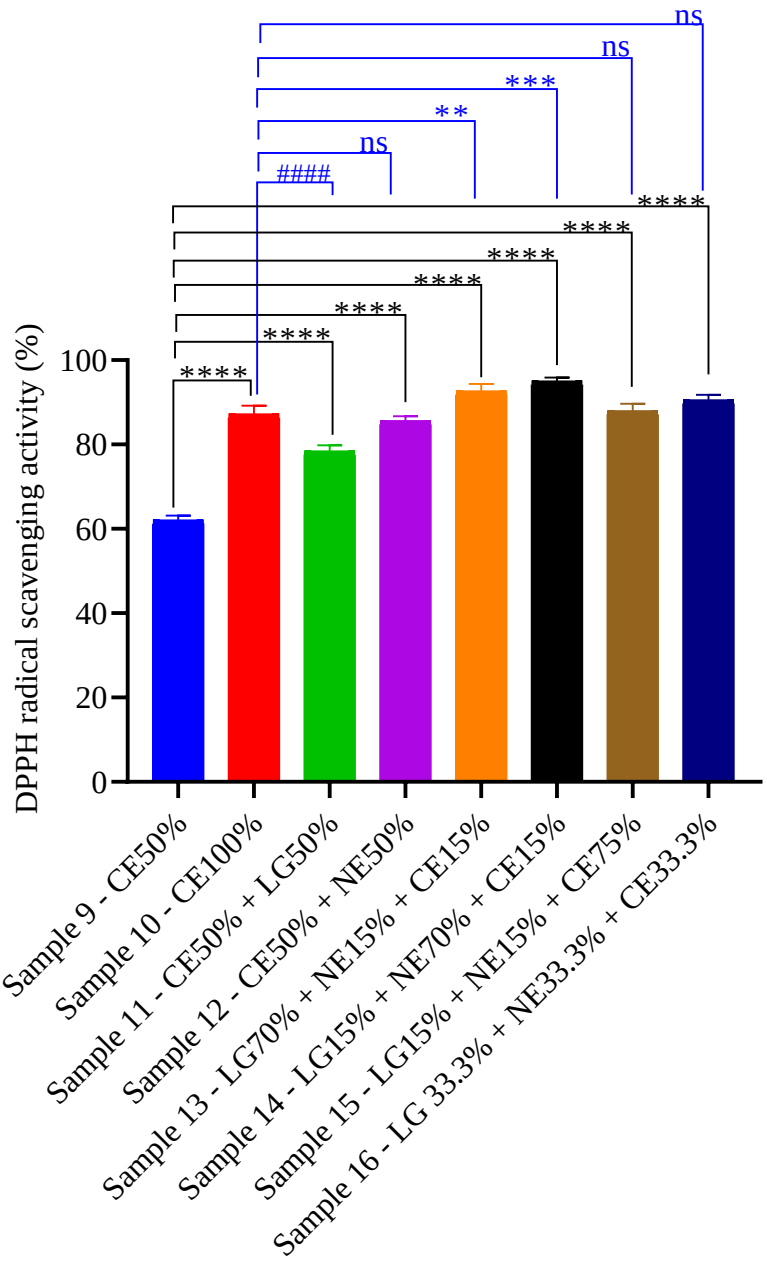

Figure 4: DPPH radical scavenging activity of Curcuma extract with Lemon grass extract, and Neem leaves extract.

Note: *CE – curcumin extract; LG – lemon grass extract; NE – neem leaves extract. **** - p<0.0001; *** - p<0.001; ** - p<0.01; * - p<0.05; ns – non-significant.*

4. CONCLUSION

In this research study substances like turmeric rhizomes (*Curcuma longa*), neem leaves (*Azadirachta indica*), and lemongrass leaves (*Cymbopogon citratus*) have been utilized to test their individual and synergistic antioxidant activity, DPPH radical scavenging assay for

single plant and combined plant extracts was carried out. Two or three plant combinations showed synergistic interactions in the DPPH radical scavenging assay. Some plant combinations showed increased antioxidant activity compared to single plant extract, but the result was non-significant, and some plant combinations showed significant antioxidant activity. Some combinations of an equal mixture of two or three plant extracts exhibited antagonistic interactions. So, in our opinion combinations of plant extracts having antagonistic activity should be avoided and combinations having synergistic activity should be used for antioxidant purposes.

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6. DECLARATION

The authors have no conflict of interest.

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