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Research Article

ENHANCEMENT OF *IN VITRO* ANTIOXIDANT POTENTIAL OF *TERMINALIA CHEBULA* BY VARIOUS FRUIT EXTRACTS AND OPTIMIZATION OF CONCENTRATION BY RESPONSE SURFACE METHODOLOGY

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ABSTRACT

Objective: The main objective of this study was to determine the enhancement of *in vitro* antioxidant potential of fruits of *Terminalia chebula* (TC) when used in combination with fruit extracts of *Phyllanthus emblica, Ananas comosus, and Punica granatum*. Hydroxyl (OH) radical scavenging and 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assays were used to analyze the antioxidant potential.

Method: Formulations of different combinatorial concentrations of fruits to prepare a mixture were achieved with central composite design through response surface methodology. Screening of 300 different combinations of various concentrations was done through hydroxyl radical scavenging assay followed by statistical analysis of data. Further validation of results was done by measuring the antioxidant potential of most bioactive extracts by DPPH method.

Results: Screening of 300 samples of different combinations for antioxidant potential revealed the samples with highest percentage inhibition in aqueous (85.2%), ethanolic (92.9%), and aqueous-ethanolic (84.21%) extracts. Data were subjected to analysis of variance and generated a three-dimensional response surface plot for highest activity. Further subjecting these extracts to DPPH assay revealed a significant enhancement in the antioxidant potential of ethanolic extract of TC when used in mixture with other plants.

Conclusion: Antioxidant activity of TC was enhanced when used in combination with other fruits extracts. These synergistic studies generating valuable interactions between various phytochemicals could lead to a momentous increase in other associated activities to fight against diseases such as cancer and cardiovascular disorders. Further research on isolation of bioactive compounds in the mixture and their potential to fight various types of cancer could lead to a significant augmentation in the activity of natural compounds.

Keywords: *Terminalia chebula*, Response surface methodology, Central composite design, 2,2-diphenyl-1-picrylhydrazyl, Antioxidant potential.

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INTRODUCTION

Plants and their products are the nature's gift to humans and animals that help them to flourish by leading a disease free healthy life. Since thousands of years, plants are playing important role in maintaining human health and quality of life. The various native systems such as Siddha, Ayurveda, Unani, and Allopathy use numerous plant species to treat different ailments [1]. Medicinal plants have been used for centuries as remedies for human diseases owing to the presence of certain components of therapeutic value. The restorative properties of medicinal plants are attributable to the presence of various bioactive phytochemicals which may enlighten their conventional uses against various ailments [2].

The increased consumption of natural antioxidants present in fruits and vegetables boosts up the antioxidant capacity of plasma, and these constituents are reported to mitigate the damage caused by the oxidative stress [3-5]. As revealed by the latest studies, the defensive effect of fruits and vegetables is at least partially attributable to the phytochemicals present in them [6-8]. The additive and synergistic effects of phytochemicals in fruits and vegetables have been proposed to be responsible for their potent antioxidant and anticancer potentials. The benefit of a diet rich in fruits and vegetables is credited to the complex mixture of phytochemicals present in these and other whole foods [9-11].

To protect the cells and organ systems of the body against reactive oxygen species, humans have evolved a highly sophisticated and complex antioxidant protection system that functions interactively and synergistically to neutralize free radicals. Under natural conditions, a dynamic balance between the free radicals and antioxidants is maintained. The amount of antioxidant principles present under normal physiological conditions may be insufficient to neutralize free radicals generated. Therefore, antioxidants can be given from external sources like food, etc., to protect against harmful diseases and to prevent, delay, or ameliorate many of these disorders [12].

The present research has been designed to study the synergistic effect of a common herb, *Terminalia chebula* (TC) over the antioxidant potential of various fruit extracts. TC belongs to the family Combretaceae and is found throughout India, especially in deciduous forests and areas of light rainfall [13]. In Tibet, due to its astonishing wound healing property, it is entitled as "the king of medicines". So far, literature shows that TC have potential biological and pharmacological properties including antibacterial, antimutagenic, antiviral, antifungal, adaptogenic, anti-anaphylactic, hypocholesterolemic, gastrointestinal motility improving, anti-ulcerogenic, hepatoprotective, cardioprotective, radioprotective, antidiabetic, retinoprotective, purgative, wound healing, antispasmodic, immunomodulatory, and chemopreventive potential [14]. From the above properties of plant, it can be speculated that when it will be used in combination with other fruit extracts, it will enhance the antioxidant and anticancer potential of the extract to a greater extent and this mixture could be a potent anticarcinogenic agent.

METHODS

Chemicals and reagents

2,2-diphenyl-1-picrylhydrazyl (DPPH), thiobarbituric acid (TBA), trichloroacetic acid (TCA), ascorbic acid, and ethylenediaminetetraacetic

acid (EDTA) were purchased from Sigma-Aldrich, USA, whereas hydrogen peroxide and 2-deoxyribose were procured from HiMedia Lab Ltd., Mumbai, India. Distilled deionized water was prepared by Camco Industries, Chandigarh (India).

Plant sample collection and authentication

Phyllanthus emblica (PE)*, Ananas comosus* (AC), *Punica granatum* (PG), *and* TC used in the present investigation were procured from local market of Chandigarh, India. Botanical identification of above-mentioned samples was done at Botany Department, Punjab University, Chandigarh. The deposition of voucher specimens in herbarium was done with accession number 21126, 21127, 21128, and 21129 for TC, PG, AC, and PE, respectively.

Extract preparation

Aqueous, ethanolic, and aqueous-ethanolic (1:1) extracts of fruits of TC, PG, AC, and PE were prepared by the method described by Chintalapani *et al*., 2018, by doing slight modifications as discussed ahead [15]. Fruits were dried at 40° for 3–4 days and powdered. Extract preparation of four samples was achieved by overnight soaking the dried powder in a respective solvent with a ratio of 1:10 (w/v) subjected to intermittent shaking. Furthermore, extracts were subjected to evaporation under reduced pressure in a rotary evaporator at 40°C and stored at 4°C for further analysis.

Optimization of sample concentrations using response surface methodology (RSM)

In the present experimental set, central composite design (CCD) was explored for making various combinations of different concentration of three fruit extracts, whereas the concentrations of TC (50, 100, 200, 400, and 500 µg/ml) were fixed for each set. The independent variables were PE: 50–300 µg/ml, AC: 50–200 µg/ml, and PG: 20–200 µg/ml. The subjection of above-mentioned concentration range of independent variables in CCD generated 20 combinations by design expert software for analysis of antioxidant potential with respect to selected concentrations of TC for each set.

***In vitro* antioxidant assay**

Hydroxyl radical (OH) scavenging activity

Deoxyribose assay was used to determine the ability of extract to scavenge the hydroxyl radical generated from $Fe3^{+}$/Ascorbate/EDTA/H_2O_2 system in aqueous medium. Reaction mixture containing 3.0 mM deoxyribose (100 µl), 0.1 mM $FeCl_3$ (200 µl), 0.1 mM EDTA (200 µl), 0.1 mM ascorbic acid (100 µl), 1 mM hydrogen peroxide (H_2O_2) (100 µl), and 20 mM phosphate buffer (pH 7.4) was subjected to 20 different combinatorial concentrations of extracts (500 µl) as generated by RSM. This assay was run in five sets, each set having a fixed concentration of TC, 50,100,200,400, and 500 µg/ml.

Followed by an incubation of 1 h at 37°C, the amount of TBA -reactive substance formed due to the attack of hydroxyl radicals on the deoxyribose was measured as per the method described by Ohkawa *et al*. [16]. Thereafter, 1 ml of TBA, 1% and 1 ml of TCA, 2.8% were added in the reaction mixture followed by incubation at 100°C for 20 min. L-ascorbic acid was employed as standard reference. The pink color thus formed in reaction mixture was measured at 532 nm against a blank containing deoxyribose and buffer. The percentage inhibition was calculated by the following equation:

Inhibition of lipid peroxidation (%) = $(A_{control}-A_{sample})/A_{control}\times100$

DPPH radical scavenging assay

The screened combinations were subjected to DPPH free radical scavenging activity according to slight modifications in the protocol [17]. Briefly, 3.5 ml methanolic solution of 0.5 mM DPPH was added to 80 µl of various combinatorial concentrations of plant extract followed by incubation at 37°C for 20 min in the dark. Methanol was used as a blank. However, antioxidant activity of L-ascorbic acid as standard reference was also assayed. The scavenging activity of the extract was estimated by the following equation, where A denotes absorbance:

% inhibition = $(A_{control}-A_{sample})/A_{control}\times100$

The inhibitory concentration (IC_{50}) values were calculated using the dose inhibition curve in linear range by plotting the extract concentration versus the corresponding scavenging effect in terms of % inhibition.

RESULTS AND DISCUSSION

Response for optimization

Improving the process conditions for the achievement of a desired product with high yield, low production costs, and low use of energy requires the use of suitable optimization procedures. RSM software generated 20 different combinations of various concentrations with respect to PE, AC, *and* PG fruit extracts as represented in Table 1. These 20 combinations were studied to impose an enhancing effect on the *in vitro* antioxidant potential of TC. Each selected concentration of TC (50, 100, 200, 400, and

Table 1: The 20 different combinations generated by RSM. Factors 1, 2, and 3 correspond to the different concentrations of PE, AC, and PG, respectively. Run represents combination number from C1 to C20

Run	Factor 1 A:P. emblica microgram/ml	Factor 2 B:A. comosus microgram/ml	Factor 3 C:P. granatum microgram/ml
1	50	200	20
2	175	251.134	110
3	175	125	110
4	175	125	110
5	175	125	110
6	50	50	20
7	300	200	200
8	175	125	110
9	50	200	200
10	385.224	125	110
11	50	50	200
12	175	125	41.3614
13	300	50	20
14	175	125	110
15	175	125	261.361
16	35.2241	125	110
17	175	125	110
18	175	1.13445	110
19	300	200	20
20	300	50	200

RSM: Response surface methodology, PE: *Phyllanthus emblica*, AC: *Ananas comosus*, PG: *Punica granatum*

500 µg/ml) was assayed individually with 20 combinations generated by RSM. Response for the optimization of concentration was analyzed in terms of percentage inhibition of lipid peroxidation. Similar studies were done by the use of CCD to study the effect of pectinase concentration, cellulase concentration, hemicellulase concentration, temperature, and incubation time on the stevioside extraction from *Stevia rebaudiana* leaves. The authors tested 26 experimental conditions and optimized them using graphical and numerical approaches [18].

Hydroxyl radical (OH) scavenging assay

Quantification of hydroxyl radical scavenging activity of 20 different combinations was achieved by measuring the inhibition of the degradation of 2-deoxyribose by the free radicals produced by the Fenton reaction. All combinations revealed a wide variation in the antioxidant potential for aqueous, ethanolic, and aqueous-ethanolic extracts. Whereas, the highest response in enhancing the antioxidant potential of TC was shown by combination number 15 of ethanolic extract (92.9%) containing PE, AC, PG, and TC in 175, 125, 261.3, and 400 µg/ml concentration, respectively, followed by combination number 10 for aqueous (85.2%) containing TC 400 µg/ml and combination number 16 for aqueous-ethanolic extracts (84.21%) containing TC 500 µg/ml. The hydroxyl radical has the capacity to break DNA strands contributing to carcinogenesis, mutagenesis, and cytotoxicity. Moreover, it is considered one of the quick initiators of lipid peroxidation process [19]. Tables 2-4 represent the complete result of hydroxyl (OH) radical scavenging assay for all the five concentrations of TC: 50, 100, 200, 400, and 500 µg/ml with respect to aqueous, ethanolic, and aqueous-ethanolic extracts.

Statistical analysis of data

Hydroxyl radical scavenging assay was performed on a total of 300 different combinatorial concentrations, 100 each of aqueous, ethanolic, and aqueous-ethanolic extracts. Results were calculated in terms of percentage inhibition of hydroxyl radical by various concentrations of extracts, and highest value of percent inhibition corresponds to ethanolic combination number 15 (92.98%) with the concentration of TC being 400 µg/ml. Statistical significance of model that generated values of combination 1–20 (Table 1) for ethanolic extract containing TC 400 µg/ml was checked by F-test analysis of variance as represented in Table 5. The Model F-value of 3.42 implies that the model is significant. $p<0.0500$ indicates that the model terms are statistically significant. The lack of fit F-value of 3.43 implies that the lack of fit is not significant relative to the pure error. This is in agreement with a study in which RSM was employed to optimize the conditions for antioxidant potential and polyphenols from apricot powder (*Prunus armeniaca* L.) using four independent variables: Methanol (20%, 35%, 50%, 65%, and 80%), solvent/sample ratio (10, 15, 20, 25, and 30), temperature (20, 30, 40, 50, and 60°C), and time (20, 30, 40, 50, and 60 min). The results showed that antioxidant potential and total polyphenols in the experiments varied from 76.15% to 96.68% and 8.77 to 12.11 mg GAE/g, respectively. The F-values for antioxidant potential and total polyphenols were 0.99 and 4.44, respectively [20]. The validation of the results of analyzed data in the present investigation can be drawn from three-dimensional response surface plot generated by design expert software (Trial Version 11), Stat Ease Inc., USA, as shown in Fig. 1. The graph is plotted between various concentrations of ethanolic extracts in combinatorial concentrations and percentage scavenging of OH radicals. The values from the graph represent that the OH radical scavenging by the various combinations of extracts is not dependent on the increasing or decreasing concentrations of extracts.

DPPH radical scavenging assay

Extensive use of DPPH radical scavenging activity has been observed for screening antioxidants from fruit juices or extracts [21]. In a study done to understand synergetic antioxidant effect of several common natural pigments among the gardenia yellow, black bean red, and sorghum red, synergetic effect and optimal formulation of compound antioxidant were determined with the RSM. The total scavenging rate was 66.78%, and it was less than the combined formula slightly. It showed that combined formula antioxidant activity was better than single antioxidant and consistent with the regression model analysis [22]. In another study, the antioxidant activity of whole plant extracts of *Sesuvium portulacastrum* L. was checked by DPPH assay in which diethyl ether extract showed the highest total phenolic content and antioxidant potential among all the extracts of *S. portulacastrum* [15]. In the present study, the results of DPPH radical scavenging activity of ethanolic extract combination number 15 increased with increase in concentration representing a dose-dependent curve as shown in Figs. 2-4. The antioxidant and radical scavenging properties of plants are based on their medicinal value. Results in this study coincide with the results of ethanolic extracts of *Tribulus terrestris* fruits (TTFs) and *Mesua ferrea* flower (MFF), both of which exerted a significant

Table 2: Percentage inhibition of hydroxyl radical by phytochemicals present in the aqueous extract of PE, AC, PG, and various concentrations of TC. The aqueous combination number 10 with TC=400 µg/ml showed the highest percentage inhibition of OH radical (85.25%)

Hydroxyl radical (OH) scavenging assay of aqueous extract					
Combination number	**% inhibition with TC (50 µg/ml)**	**% inhibition with TC (100 µg/ml)**	**% inhibition with TC (200 µg/ml)**	**% inhibition with TC (400 µg/ml)**	**% inhibition with TC (500 µg/ml)**
1	68.31	61.75	69.95	78.69	80.33
2	74.32	65.03	73.77	76.50	81.97
3	61.75	62.30	78.14	83.61	77.60
4	64.48	68.85	75.41	78.14	84.70
5	66.67	61.75	68.85	79.78	81.97
6	61.20	56.28	74.86	84.15	79.23
7	62.30	63.93	71.04	70.49	77.60
8	63.39	71.04	67.21	72.68	75.96
9	54.10	62.30	68.31	70.49	75.96
10	60.66	63.93	76.50	85.25	79.23
11	58.47	63.39	75.96	73.22	77.05
12	69.95	63.39	76.50	74.86	83.06
13	72.68	67.21	78.69	82.51	73.22
14	63.39	63.39	78.14	75.96	74.86
15	65.57	72.68	77.60	76.50	74.32
16	51.91	80.33	80.33	68.31	69.95
17	66.12	70.49	78.69	71.58	81.42
18	67.21	69.40	76.50	76.50	79.78
19	69.40	70.49	78.14	79.78	73.77
20	73.22	71.58	79.23	81.97	78.69

PE: *Phyllanthus emblica*, AC: Ana*nas comosus*, PG: *Punica granatum*, TC: *Terminalia chebula*

Table 3: Percentage inhibition of hydroxyl radical by phytochemicals present in the ethanolic extract of PE, AC, PG, and various concentrations of TC. The ethanolic combination number 15 with TC=400 µg/ml showed the highest percentage inhibition of OH radical (92.98%)

Hydroxyl radical (OH) scavenging assay of ethanolic extract					
Combination number	**% inhibition with TC (50 µg/ml)**	**% inhibition with TC (100 µg/ml)**	**% inhibition with TC (200 µg/ml)**	**% inhibition with TC (400 µg/ml)**	**% inhibition with TC (500 µg/ml)**
1	82.46	77.78	71.93	67.25	83.04
2	77.78	76.61	79.53	75.44	85.96
3	73.68	82.46	77.78	67.84	79.53
4	79.53	78.36	75.44	64.91	73.68
5	75.44	80.12	81.87	70.18	72.51
6	77.78	84.21	83.04	60.23	83.63
7	72.51	70.76	71.35	61.40	80.70
8	55.56	72.51	75.44	63.16	83.04
9	61.99	70.76	85.38	55.56	84.21
10	66.67	84.21	80.70	60.82	82.46
11	71.35	73.68	72.51	60.23	76.02
12	50.88	74.86	77.78	67.25	77.78
13	81.29	81.87	80.12	80.12	81.87
14	61.40	75.44	71.93	61.99	83.63
15	87.72	76.61	79.53	92.98	78.95
16	77.19	68.42	74.27	51.46	75.44
17	81.87	71.93	78.36	58.48	77.19
18	71.93	76.02	76.61	66.08	80.70
19	60.23	79.78	78.36	67.25	77.78
20	69.59	80.70	83.04	71.35	79.53

PE: *Phyllanthus emblica*, AC: *Ananas comosus*, PG: *Punica granatum*, TC: *Terminalia chebula*

Table 4: Percentage inhibition of hydroxyl radical by phytochemicals present in the aqueous-ethanolic extract of PE, AC, PG ,and various concentrations of TC. The aqueous-ethanolic combination number 16 with TC=500 µg/ml showed the highest percentage inhibition of OH radical (84.21%)

Hydroxyl (OH) radical scavenging assay of aqueous-ethanolic extract					
Combination number	**% inhibition with TC (50 µg/ml)**	**% inhibition with TC (100 µg/ml)**	**% inhibition with TC (200 µg/ml)**	**% inhibition with TC (400 µg/ml)**	**% inhibition with TC (500 µg/ml)**
1	64.91	73.68	60.53	37.72	64.04
2	77.19	66.67	65.79	33.33	76.32
3	77.19	78.07	64.04	32.46	74.56
4	78.07	31.58	60.53	39.47	76.32
5	50.00	42.98	57.89	38.60	49.12
6	70.18	52.63	66.67	48.25	69.30
7	81.58	54.39	70.18	40.35	80.70
8	70.18	42.98	52.63	43.86	71.05
9	56.14	39.47	16.67	55.26	57.89
10	66.67	37.72	61.40	40.35	67.54
11	50.00	57.02	53.51	48.25	51.75
12	33.33	41.23	57.89	41.23	33.33
13	25.44	35.96	50.00	39.47	25.44
14	70.18	21.93	57.02	38.60	70.18
15	61.40	48.25	64.04	42.98	60.53
16	81.58	14.91	43.86	44.74	84.21
17	70.18	20.18	50.00	38.60	68.42
18	3.51	56.14	56.14	48.25	4.39
19	70.18	23.68	57.02	44.74	69.30
20	45.61	14.91	55.26	45.61	48.25

PE: *Phyllanthus emblica*, AC: *Ananas comosus*, PG: *Punica granatum*, TC: *Terminalia chebula*

scavenging activity on the DPPH radical which was found to be increasing with the increasing concentration and thus revealed dose-dependent curves. At 250 µg/ml concentration, the exerted values of MFF and TTF extracts were found to be 54.96% (with IC_{50} 131.0 µg/ml) and 51.46% (with IC_{50} 142.27 µg/ml), respectively, when compared to ascorbic acid, the standard (86.10%) [23]. Whereas, in the present study the highest activity was found in ethanolic extract, followed by aqueous and aqueous-ethanolic extract. The DPPH scavenging activity of TC was enhanced from 72.36% to 87.37% when used in combination with other extracts. The IC_{50} DPPH values for ascorbic acid, aqueous, ethanolic, aqueous-ethanolic, and TC extracts were obtained from the linear regression equation from respective graphs as represented in Table 6.

A significant enhancement in the antioxidant potential of TC when used in a mixture with other extracts is supported by the study which states that several plant preparations such as a mixture of aqueous extracts of *Spilanthes Africana*, *Portulaca oleracea,* and *Sida rhombifolia* are currently utilized in Foumban (West Cameroon) to manage diabetes that was induced to rats by intraperitoneal injection of streptozotocin at a dose of 50 mg/kg b.w. Each extract and the mixture demonstrated a significant scavenging property on DPPH and OH radicals and revealed

Table 5: The ANOVA for quadratic model for percentage inhibition of OH radical (generated by design expert, RSM). The p value of the lack of fit value was of 0.1013 (not significant), the model was of 0.0342 (significant), which showed that the model fit the RSM data significantly

Source	Sum of square?	df	Mean^2	F-value	p	
Model	1197.73	9	133.08	3.42	0.0342	Significant
A-P. emblica	343.86	1	343.86	8.84	0.0140	
B-A. comosus	1.83	1	1.83	0.0471	0.8325	
C-P. granatum	22.90	1	22.90	0.5890	0.4605	
AB	79.04	1	79.04	2.03	0.1844	
AC	1.07	1	1.07	0.0275	0.8716	
BC	9.62	1	9.62	02474	0.6297	
A^2	392.20	1	392.20	10.09	0.0099	
B^2	21.03	1	21.03	0.5410	0.4789	
C^2	618.78	1	618.78	15.91	0.0026	
Residual	388.80	10	38.88			
Lack of fit	300.97	5	60.19	3.43	0.1013	Not significant
Pure error	87.83	5	17.57			
Cor total	1586.54	19				

RSM: Response surface methodology, ANOVA: Analysis of variance

Table 6: The results of DPPH assay for concentrations ranging from 5, 10, 15, and 20 µg/ml for ascorbic acid, aqueous, ethanolic, aqueous-ethanolic, and TC extracts. Comparison of IC_{50} values and percentage inhibition of DPPH radical by TC and various combinatorial extracts. C10, C15, and C16 represent combinations with concentration of TC being 400, 400, and 500 µg/ml, respectively. Values are mean of three replicates ± SD

Sample concentration (µg/ml)	DPPH free radical scavenging activity (%)				
	Ascorbic acid (AA)	Aqueous extract (C10 + TC 400 µg/ml)	Ethanolic extract (C15 + TC 400 µg/ml)	Aqueous-ethanolic (C16 + TC 500 µg/ml)	TC (TC 500 µg/ml)
5	23.59±2.95	19.53±1.24	27.50±1.80	20.33±1.80	18.45±1.84
10	38.25±2.19	32.67±1.45	41.10±2.32	31.16±2.81	28.32±2.34
15	56.73±2.51	53.42±1.48	64.10±1.62	50.60±2.52	52.64±2.15
20	76.73±2.45	72.63±2.06	87.37±1.94	70.68±2.89	72.36±2.25
IC_{50} (µg/ml)	12.93	14.01	11.41	14.50	14.37

SD: Standard deviation, TC: *Terminalia chebula*, DPPH: 2,2-diphenyl-1-picrylhydrazyl, IC_{50}: inhibitory concentration

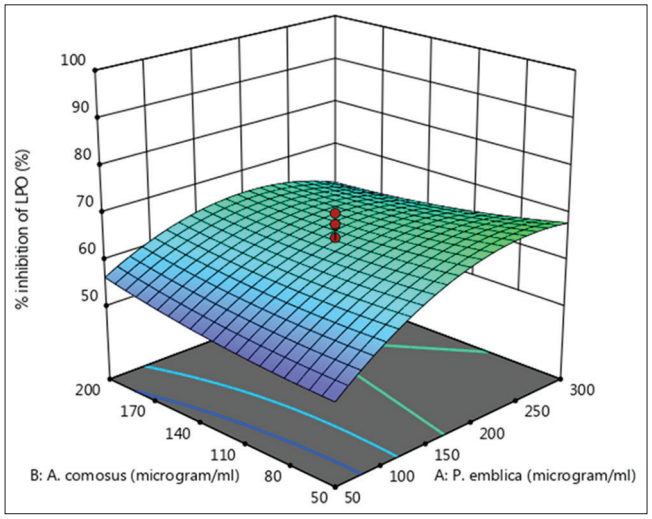

Fig. 1: Three-dimensional response surface plot for establishing the response values between the independent variables represented by A and B and the response called inhibition of lipid peroxidation by OH radical through various extracts

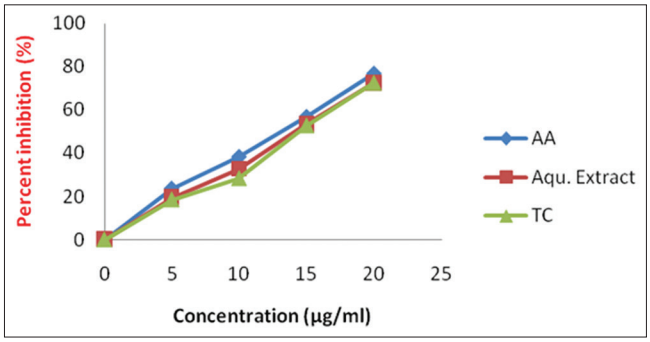

Fig. 2: Comparison of 2,2-diphenyl-1-picrylhydrazyl scavenging activity of aqueous extract combination (C10+ *Terminalia chebula* [TC] 400 µg/ml), only TC (500 µg/ml) and ascorbic acid as standard. The IC50 value of aqueous extract of combination C10+TC=400 µg/ml is calculated to be 14.01 µg/ml with the help of linear regression equation being y=3.582 x-0.182 and the value of R^2=0.995

a good antioxidant property. Thus, it can be concluded from the study that the mixture of plant extracts had hypoglycemic, antioxidant, and hypolipidemic properties and can be used for the management of diabetes mellitus [24].

CONCLUSION

The present study indicates that a mixture of PE, AC, PG, and TC in fixed concentration exhibits a significant amount of antioxidant potential that is higher than the potential of TC alone. Generation of 300 different combinations with varying concentrations was achieved by application of CCD through RSM software. This observation highlights the importance of selecting the best combination of extract based on

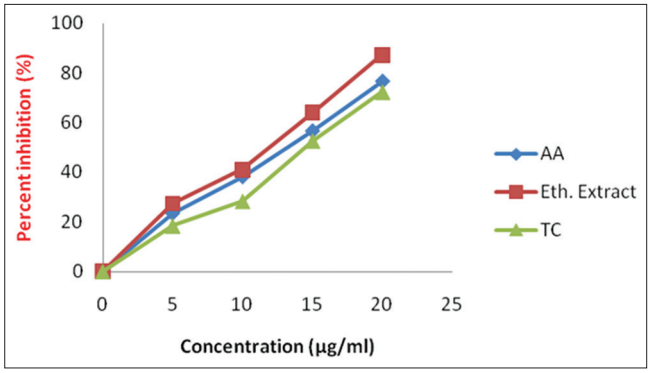

Fig. 3: Comparison of 2,2-diphenyl-1-picrylhydrazyl scavenging activity of ethanolic extract combination (C15+ *Terminalia chebula* [TC] 400 µg/ml), only TC (500 µg/ml) and ascorbic acid as standard. The IC50 value of ethanolic extract of combination C15+TC=400 µg/ml is calculated to be 11.41 µg/ml with the help of linear regression equation being y=4.226x+1.744 and the value of R2=0.992

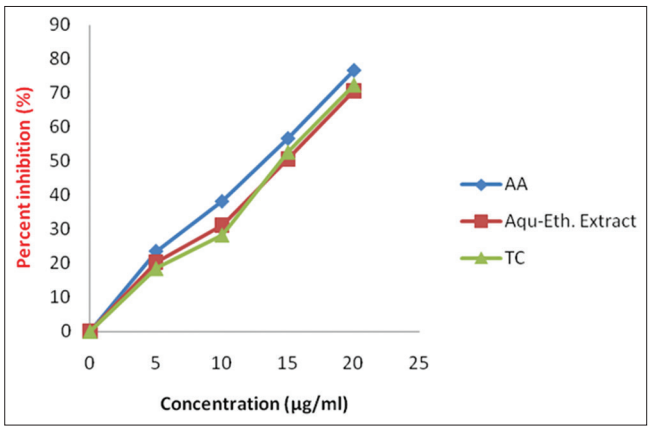

Fig. 4: Comparison of 2,2-diphenyl-1-picrylhydrazyl scavenging activity of aqueous-ethanolic extract combination (C16+ *Terminalia chebula* [TC] 500 µg/ml), only TC (500 µg/ml) and ascorbic acid as standard. The IC50 value of aqueous-ethanolic extract of combination C16+TC=500 µg/ml is calculated to be 14.50 µg/ml with the help of linear regression equation being y=3.432x-0.228 and the value of R^2=0.991

the results of hydroxyl radical scavenging assay followed by statistical analysis of generated data. The most potent bioactive combinations screened through above method were subjected to DPPH radical scavenging assay. The outcome arising from this study revealed a significant enhancement in the antioxidant potential of TC when used in a mixture. These *in vitro* interactions of bioactive compounds of a mixture provide interesting information on enhancing antioxidant potential by synergistic effects. However, further work on combinatorial studies of these extracts may generate an enhanced potential to cure oxidative stress-related diseases such as cancer, cardiovascular, and other chronic diseases.

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AUTHOR'S CONTRIBUTION

All the authors have equally contributed.

CONFLICTS OF INTEREST

The authors declared that they have no conflicts of interest.

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