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In Vitro Evaluation of Antioxidant Activity of Padikara Parpam (PP), L-Ascorbic Acid (LAA), and combination of Padikara Parpam and L-Ascorbic Acid (PPLAA)

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ABSTRACT
The antioxidant activity of Padikara Parpam (PP), L-Ascorbic Acid (LAA) and their combination Padikara Parpam and L-Ascorbic Acid (PPLAA) was assessed in this study performing various in vitro tests. The antioxidant activity of these three different test samples PP, LAA and PPLAA was assessed using TAC, DPPH assay, hydrogen peroxide, nitric oxide scavenging activity, estimation of lipid peroxidation using egg yolks, ABTS Radical Cation, superoxide radical, and deoxyribose radical scavenging activity, β carotene linoleic acid assay, and assay of superoxide dismutase (SOD). The findings indicated that all three compounds demonstrated dose-dependent free radical scavenging, with PPLAA showing the highest activity at 500 µg/mL, particularly against hydrogen peroxide and DPPH (83% inhibition). The compounds effectively inhibited lipid peroxidation, superoxide and nitric oxide radicals, and β-carotene bleaching, demonstrating their capacity to reduce oxidative stress. The antioxidant capability of PP, LAA, and their combination (PPLAA) is demonstrated in this work using in vitro techniques. These findings support the use of them in formulations meant to lessen oxidative stress and associated illnesses that can act as therapeutic agents.
Keywords: *Padikara Parpam (PP), L-Ascorbic Acid (LAA), Combination of Padikara Parpam and L-Ascorbic Acid (PPLAA), Antioxidant Acivity*

INTRODUCTION

Pathophysiology of many chronic diseases, such as cancer, cardiovascular disease, diabetes, and neurodegenerative diseases, is significantly influenced by oxidative stress, which is a major cause of cellular damage. An inadequate antioxidant defence system and excessive reactive oxygen species (ROS) generation are the causes of this imbalance.

Antioxidants are compounds that counteract reactive oxygen species (ROS) and stop oxidative damage to biomolecules like DNA, proteins, and lipids. Finding possible therapeutic agents requires assessing the antioxidant capabilities of both synthetic and natural substances. The antioxidant activity of plant extracts may be explained by the presence of certain phytochemicals.(1)

By preventing, stopping, and delaying oxidative processes brought on by free radicals, antioxidants are essential substances that contribute to human protection.(2) Because of their special ability, antioxidants are being used more and more to regulate reactive oxygen species. Antioxidants derived from plants shield the body from a variety of illnesses by preventing the action of free radicals.

Due to their many benefits, drug combinations are used to treat infections and illnesses. Combinations of medications broaden the spectrum of antibiotics to treat more illnesses, which is crucial for serious infections. Researchers can prevent needless negative synergies as well as identify new phytomedicines or pharmacological combinations by studying synergistic interactions. Combinations of medications can aid in the eradication of drug resistance.(3)

Hence, in this study, the antioxidant activity of three different test samples—Padikara Parpam (P), L-Ascorbic Acid (LAA), and combination of both Padikara Parpam and L-Ascorbic Acid (PPLAA)—was assessed using both TAC and DPPH assays at varying concentrations. Ascorbic acid was used as a standard antioxidant for comparison.

MATERIALS AND METHODS

Total Antioxidant Activity Assay

The combined antioxidant capacity of PP, LAA, and PPLAA was evaluated.(4) The total antioxidant capacity (TAC) reagent was created in this manner: Sulfuric acid (7.45 ml of 0.6 mM), sodium sulphate (0.9942 g of 28 mM), and ammonium molybdate (1.2356 g of 4 mM) were all dissolved in 250 ml of distilled water. The different drug concentrations of 100, 200, 300, 400, and 500 µg/ml were dissolved in 3 ml of TAC reagent. For ninety minutes, the reaction mixture was incubated at 95°C. A spectrophotometer was used to analyze all samples at 695 nm.(5,6,7)

DPPH Radical Scavenging Assay

Yen and Chen's 1995 technique was used to evaluate the DPPH radical scavenging activity of PP, LAA, and PPLAA.(8) In short, 2.0 ml of a 0.16 mM methanolic solution of DPPH was mixed with 2.0 ml of samples at concentrations of 100, 200, 300, 400, and 500 µg/ml. After a minute of rigorous mixing, the mixture was put aside. 30 minutes in the dark at a temperature of 25 to 28°CEach sample solution's absorbance was measured at 517 nm.(9)

Hydrogen peroxide scavenging activity

The hydrogen peroxide scavenging assay was carried.(10) A hydrogen peroxide (H_2O_2, 10 mM) solution was made in phosphate buffer (0.1 M, pH 7.4). Phosphate buffer solution (3.4 ml), H_2O_2 solution (0.6 ml), and 1.0 ml of PP, LAA, and PPLAA at concentrations of 100, 200, 300, 400, and 500 µg/ml comprised the subsequent solution. The absorbance value of the reaction mixture at 230 nm was determined following ten minutes of room temperature incubation. The sodium phosphate buffer in the blank solution does not contain H_2O_2.(11,12) H_2O_2 and a control solution with buffer were employed.

Nitric oxide scavenging activity

It was utilized by the subsequent process. 10 mM sodium nitroprusside (3 ml) was mixed with 1.0 ml of PP, LAA, and PPLAA at concentrations of 100, 200, 300, 400, and 500 µg/ml in phosphate buffer solution (pH 7.4), and the mixture was then incubated for 180 minutes at 25°C. Griess reagent was prepared just before use by combining a corresponding amount of sulfanilamide (1%) and naphthylethylene diaminedihydrochloride (1%) with 2.5% phosphoric acid. The test solution and freshly prepared Griess reagent were mixed in an equal proportion. At 546 nm, the absorbance was computed. Drug inhibition percentage was calculated and noted.(13)

Estimation of lipid peroxidation using egg yolks

A modified thiobarbituric acid- reactive species (TBARS) assay was used to measure the inhibitions of lipid peroxidation in hen eggs.(14,15) The volume of the mixture was increased to 1 ml by adding distilled water after the egg homogenate (0.5 ml, 10% distilled water) and 0.1 ml of PP, LAA, and PPLAA at concentrations of 100, 200, 300, 400, and 500 µg/ml were mixed individually. For lipid peroxidation, 0.05ml of $FeSO_4$ was added to the mixture and incubated for 30 minutes. Then, SDS (1.1%), acetic acid (1.1%), 1.5% TBA (w/v), and 0.05 ml TCA were added, mixed thoroughly, and heated in a boiling water bath for 60 minutes. Each tube was filled with 5.0 ml of butanol after chilling, and it was centrifuged for 10minutes at 3000 rpm. The absorbance was determined at 532 nm.

ABTS Radical Cation Scavenging Assay

The ABTS radical scavenging activity of PP, LAA, and PPLAA was assessed using the ABTS radical scavenging test.(16) The ABTS radical cation was made by mixing 20 mM ABTS solution with 70 mM potassium peroxodisulphate, and it was then allowed to stand at room temperature in the dark for 24 hours. After 10 minutes of incubation, the absorbance of solutions containing 0.6 ml of extract and 0.45 ml of ABTS reagent was measured at 734 nm.(17) Additionally, measurements were taken from a control tube that just had chemicals.

Superoxide radical scavenging activity

The scavenging of superoxide radicals was assayed.(18) 2.64 milliliters of phosphate buffer (50 mM, pH), 0.2 milliliters of EDTA (12 mM), 0.1 milliliters of Nitro blue tetrazolium, 0.05 milliliters of riboflavin (20 g), and 0.2 milliliters of PP, LAA, and PPLAA at concentrations of 100, 200, 300, 400, and 500 µg/ml were prepared as assay tubes. The control tube was set up using DMSO (dimethyl sulfoxide) solution in place of the test solution. The tubes were exposed to consistent fluorescent light illumination for 30 minutes following the measurement of the solutions' initial optical densities at 560 nm. A560 was measured again, and the difference in OD was used to compute the amount of superoxide production. By comparing the test samples' percentage of inhibition to the OD of the control tubes, the result was obtained.(19) A control

tube that just contained reagents was also measured.

Deoxyribose Radical Scavenging Activity

The deoxyribose hydroxyl radical scavenging activity of PP, LA, and PPLA was determined.(20) Test solutions at concentrations of 100, 200, 300, 400, and 500 µg/ml were added in aliquots to create a reaction mixture of 2.0 ml, FeSO4.7H2O, EDTA, and deoxyribose. After increasing the volume to 1.8 ml with phosphate buffer (0.1M, pH-7.4), 0.2 ml of 10mM H2O2 was added. The mixture was incubated in the dark at 37°C for 4 hours. Following incubation, 1 milliliter of 2.8% TCA and 1% TBA were added to the mixture, which was then heated for ten minutes in a water bath. At 532 nm after treatment, absorbance was measured. After filtration, the absorbance was measured if the mixture was turbid.(17) A control tube that just contained reagents was also measured.

β carotene linoleic acid assay

The β-carotene linoleic acid assay was carried out in compliance with Zargar et al. (2011).(21) In summary, 200 mg of linoleic acid, 20 mg of Tween 40, and 2 mg of β-carotene were dissolved in 10 ml of chloroform and placed in a flask. Chloroform was evaporated using a vacuum evaporator. Next, saturate it with oxygen by shaking 50 cc of pure water for 30 minutes. This mixture is used as a stock solution. 200 l of PP, LAA, and PPLAA at concentrations of 100, 200, 300, 400, and 500 µg/ml were mixed with 2.5 ml of stock solution in the test tube. After that, the samples were heated for three hours at 50°C. A470 nm, the absorbance was measured. Control was measured only with the reagents.

Assay of Superoxide Dismutase (SOD)

The Kakkar et al., 1984 technique was used to measure SOD scavenging activity.(22) The synthesis of NADH-phenazine metho sulphate nitro blue tetrazolium formazon is used as the basis for the test of SOD. The supernatant from centrifuging an accurate amount (0.5g) of PP, LAA, and PPLAA with 3.0 ml of potassium phosphate buffer was used as the enzyme for the experiment. It is possible to extract the colour created at the end of the reaction and butanol was measure at 560 nm. Sodium pyrophosphate (1.2 ml), PMS (0.1 ml), NBT (0.3 ml), and enzyme preparation (0.2 ml) were added in the test mixture, which also contained water.

The addition of 0.2 ml of NADH started the reaction. After 90 seconds of incubation at 30 degrees, the mixture was stopped by adding 1.0 cc of glacial acetic acid. Following a shake with 4.0m of n-butanol, the action mixture was left for 10 minutes and then centrifuged. The chromogen in the butanol layer was measured at 560 nm. A control tube that just contained reagents was also measured.

RESULTS

Total Antioxidant Activity

The test samples and standard ascorbic acid were taken for estimating total antioxidant activity at concentrations ranging from 100 to 500 µg/ml, and the OD values were recorded in Table 1. The standard curve for ascorbic acid, PP, LAA, and PPLAA total antioxidant activity is shown in the table. The total antioxidant activity of LAA (15.71 ± 0.28 to 67.34 ± 0.29%) indicates higher activity followed by total antioxidant activity of PPLAA has registered 14.28 ± 0.18 to 65.47 ± 0.46 whereas, PP (13.14± 0.17 to 60.66 ± 0.68%), indicates minimal inhibition when compared to LAA and PPLAA.

Table 1: Total antioxidant activity of PP, LAA and PPLAA

Conc µg/ml	PP %	LAA %	PPLAA %
100	13.14 ± 0.17	15.71 ± 0.28	14.28 ± 0.18
200	22.34 ± 0.21	28.44 ± 0.17	23.80 ± 0.25
300	30.43 ± 0.61	41.25± 0.16	32.69 ± 0.37
400	48.72 ± 0.32	47.03 ± 0.21	55.27 ± 0.14
500	60.66 ± 0.68	67.34 ± 0.29	65.47 ± 0.46
IC 50 Values (µg/mL)	303.214	291.156	303.214
Values represented mean ± SD of three independent experiments with three replicates each			

DPPH radical scavenging assay

Table 2 depicts the DPPH radical scavenging activities (%) of PP, LAA, and PPLAA at various concentrations (100 – 500 µg/ml). There were differences in the ability of these samples to scavenge DPPH, but PP, LAA and PPLAA were most effective at 500 µg/ml concentration (79.69 ± 0.66%, 78.71 ±0.94, 83.03 ± 0.75, respectively), followed by 400 µg/ml concentration of PPLAA (69.77 ± 0.353%). The least amount of DPPH radical scavenging activity was demonstrated by LAA at a concentration of 100 µg/ml (13.72 0.75%). The IC50 concentrations for PP, LAA, and PPLAA were determined to be 253.28 µg/ml, 289.78 µg/ml and 381.10 µg/ml, respectively.

Table 2: DPPH radical scavenging potential PP, LAA and PPLAA

Conc µg/ml	PP %	LAA%	PPLAA%
100	15.37 ± 0.75	13.72 ± 0.75	25.90 ± 0.46
200	33.61 ± 0.90	30.90 ± 0.61	48.83 ± 0.86
300	55.51± 1.07	51.17 ± 0.51	62.82 ± 0.75
400	67.96 ± 0.77	64.77 ± 0.96	69.77 ± 0.53
500	79.69 ± 0.66	78.71 ± 0.94	83.03 ± 0.75
IC 50 Values	289.78	381.1	253.28
Values represented mean ± SD of three independent experiments with three replicates each			

Hydrogen peroxide scavenging activity

Table 3 shows the H2O2 radical scavenging activities (%) of PP, LAA, and PPLAA at various concentrations (100 - 500 µg/ml). There were differences in the ability of these samples to scavenge H2O2, but PP, LAA and PPLAA were most effective at 500 µg/ml concentration (82.26 ± 0.17% , 78.62 ± 0.71% and 83.29 ± 0.73%, respectively), followed by 400 µg/ml concentrations of PPLAA (76.82 ± 0.62%).

The minimum amount of H2O2 radical scavenging activity was demonstrated by LAA at a concentration of 100 µg/ml (24.66 ± 0.52 %). PP, LAA, and PPLAA were found to have IC50 values of 245.14, 276.80, and 235.54 µg/ml respectively.

Table 3: Hydrogen peroxide scavenging potential of PP, LAA and PPLAA

Conc µg/ml	PP%	LAA%	PPLAA %
100	27.52 ± 0.67	24.66 ± 0.52	30.32 ± 0.81
200	42.05 ± 0.61	37.05 ± 0.61	42.09 ± 1.02
300	51.19 ± 0.23	44.19 ± 0.23	55.29 ± 1.12
400	70.18 ± 0.19	70.80 ± 0.18	76.82 ± 0.62
500	82.26 ± 0.17	78.62 ± 0.71	83.29 ± 0.73
IC 50 Values	245.14	276.8	235.54
Values represented mean ± SD of three independent experiments with three replicates each			

Scavenging activity of Nitric oxide (NO)

Nitric oxide assay of PP, LAA and PPLAA was done, and the results are shown in Table 4. The resulting OD values were used to determine the IC50 values and % of inhibition of nitric acid formation. At a concentration of 500 µg/ml, PPLAA exhibited a greater percentage of inhibition (81.89± 1.71%) which is somewhat higher than that of PP and LAA (80.25 ± 1.14 and 79.54 ± 1.43%) at the concentration of 500 µg/ml. The NO scavenging activities of PP, LAA, and PPLAA had respective IC50 values of 290.15, 2293.81, and 74.65 µg/ml.

Table 4: Nitric oxide scavenging potential of PP, LAA and PPLAA

Conc µg/ml	PP%	LAA%	PPLAA %
100	20.09 ± 0.57	22.00 ± 0.46	23.33 ± 0.52
200	32.55 ± 0.33	35.25 ± 0.27	37.12 ± 0.86
300	51.71 ± 1.15	53.67 ± 1.61	57.27 ± 0.87
400	67.10 ± 0.91	68.01 ± 1.12	70.38 ± 1.52
500	80.25 ± 1.14	79.54 ± 1.43	81.89± 1.71
IC 50 Values	290.15	293.81	274.65
Values represented mean ± SD of three independent experiments with three replicates each			

Estimation of lipid peroxidation using egg yolks

LPD scavenging activity for PP, LAA and PPLAA was done, and the results are shown in Table 5. The resulting OD values were used to determine the IC50 values and % of inhibition of LPD formation. The PPLAA sample at a concentration of 500 µg/ml demonstrated the highest inhibition of (81.89± 1.71%) which is somewhat higher than that of PP and LAA (77.37 ± 1.13% and 75.10 ± 0.1.61 %) at the concentration of 500 µg/ml. The NO scavenging activities of PP, LAA, and PPLAA had respective IC50 values of 290.15, 2293.81, and 74.65 µg/ml.

Table 5: Lipid peroxidase activity of PP, LAA and PPLAA

Conc µg/ml	PP%	LAA%	PPLAA %
100	14.61 ± 0.91	15.70 ± 0.59	18.18 ± 0.85
200	31.41 ± 0.92	30.24 ± 0.74	34.17 ± 1.11
300	45.57 ± 1.21	43.75 ± 0.66	49.90 ± 0.86
400	64.43 ± 1.22	61.13 ± 0.59	71.26 ± 0.55
500	77.37 ± 1.13	75.10 ± 1.61	80.26 ± 0. 82
IC 50 Values	298.74	310.56	292.13
Values represented mean ± SD of three independent experiments with three replicates each			

Scavenging assay of ABTS

The ABTS assay used in the current investigation demonstrated that antioxidant activity rises with concentration, with the results recorded in Table 6. At a concentration of 500 µg/ml, PP, LAA, and PPLAA showed the greatest inhibition, with percentages of 81.06 ± 0.58, 77.97 ± 0.53 and 82.64± 0.79%, respectively. At a concentration of 100 µg/ml, LAA showed the lowest level of inhibition (15.43 ± 0.98%). The IC50 values for the PP, LAA, and PPLAA lipid peroxidation assays were 290.47, 301.96, and 281.78 µg/ml, respectively.

Table 6: ABTS Radical Cation Scavenging potential of PP, LAA and PPLAA

Conc µg/ml	PP%	LAA%	PPLAA %
100	16.18 ± 0.15	15.43 ± 0.98	16.49 ± 1.14
200	35.71 ± 1.03	32.35 ± 1.12	37.05 ± 0.86
300	54.90 ± 1.45	53.04 ± 1.11	57.83 ± 0.97
400	68.26 ± 0.25	63.72 ± 0.97	71.32 ± 0.96
500	81.06 ± 0.58	77.97 ± 0.53	82.64 ± 0.79
IC 50 Values	290.47	301.96	281.78
Values represented mean ± SD of three independent experiments with three replicates each			

Scavenging activity of Superoxide radical (SO)

The results of the scavenging activity of superoxide radical tests for PP, LAA, and PPLAA are given

in Table 7. The resulting OD values were also used to determine the IC50 values and the % of inhibition. At a concentration of 500 µg/ml, the percentage of inhibition by PPLAA was discovered to be 86.70 ± 0.92%, which is maximum than that of PP and LAA (81.70 ± 1.15 and 79.11 ± 0.91%, respectively). The lowest level of scavenging activity was discovered at 100 µg/ml of LAA (19.52 ± 0.86%). The IC50 values of PP, LAA, and PPLAA for SO scavenging activity were 295.70, 323.87, and 278.09 µg/ml, respectively.

Table 7: Superoxide radical scavenging potential of PP, LAA and PPLAA

Conc µg/ml	PP%	LAA%	PPLAA%
100	24.18 ± 1.02	19.52 ± 0.86	28.14 ± 1.20
200	39.26 ± 0.88	25.36 ± 1.21	41.62 ± 1.02
300	59.37 ± 0.67	41.02 ± 0.36	65.11 ± 1.13
400	66.13 ± 0.65	60.45 ± 0.53	75.31 ± 0.56
500	81.70 ± 1.15	79.11 ± 0.91	86.70 ± 0.92
IC 50 Values	295.7	323.87	278.09
Values represented mean ± SD of three independent experiments with three replicates each			

Deoxyribose Radical Scavenging Activity

Deoxyribose assay of PP, LAA, and PPLAA was evaluated, and the results are shown in Table 8 along with IC50 values. All of the test medications' concentrations were capable of scavenging the deoxyribose radical to varying degrees, but the drug PPLAA was found to be the most effective (76.52±0.82%) at 500 µg/ml, followed by the drug PP, which showed 75.32±1.10% of inhibition. The LAA displayed the lowest deoxyribose radical scavenging potential (21.03±1.16%) at a concentration of 100 µg/ml. PP, LAA, and PPLAA had IC50 values for SO scavenging activity of 303.28, 316.32, and 281.30 µg/ml, respectively.

Table 8: Deoxyribose Radical Scavenging potential of PP, LAA and PPLAA

Conc µg/ml	PP%	LAA%	PPLAA %
100	26.16 ± 1.25	21.03 ± 1.16	27.96 ± 0.76
200	36.41 ± 1.21	33.36 ± 0.91	41.90 ±0.73
300	49.67 ± 0.91	44.13 ± 0.72	57.59 ± 0.87
400	64.22 ± 1.41	60.10 ± 0.64	66.58 ± 0.72
500	75.32 ± 1.10	73.10 ± 1.73	76.52 ± 0.82
IC 50 Values	303.28	316.32	281.3
Values represented mean ± SD of three independent experiments with three replicates each			

β carotene linoleic acid assay

Table 9 shows the scavenging capacity of PP, LAA, and PPLAA at various concentrations (100-500 µg/ml) against β carotene linoleic acid (%). The potential to scavenge carotene linoleic acid was present in all of these materials to varying degrees, however the most effective scavengers were determined to be the 500 µg/ml concentrations of PP, LAA, and PPLAA (85.87 ± 1.54, 83.67 ± 2.49 and 87.43 ± 1.57%, respectively). The lowest scavenging level of β carotene linoleic acid were demonstrated by the 100 µg/ml concentrations of PP, LAA, and PPLAA (19.42 1.48, 17.48 1.24, and 21.93 1.93%, respectively). It was revealed that the IC50 values for PP, LAA, and PPLAA were 277.31, 289.31, and 267.14 µg/ml, respectively.

Table 9: β carotene linoleic acid assay of PP, LAA and PPLAA

Conc µg/ml	PP%	LAA %	PPLAA %

100	19.42± 1.48	17.48 ± 1.24	21.93 ± 1.93
200	39.41 ± 1.07	35.64 ± 1.41	42.56 ± 2.38
300	60.13 ± 1.11	57.56 ± 1.34	62.52 ± 2.35
400	73.47 ± 1.81	72.89 ± 2.45	76.41 ± 2.95
500	85.87 ± 1.54	83.67 ± 2.49	87.43 ± 1.57
IC 50 Values	277.31	289.31	267.14
Values represented mean ± SD of three independent experiments with three replicates each			

SOD radical scavenging activity

In this study, PP, LAA, and PPLAA were the targets of the SOD radical scavenging activity. PPLAA, at a concentration of 500 µg/ml, demonstrated the highest level of inhibition (83.41 ± 1.08%), followed by the scavenging activity of PP with inhibition of 80.16 ± 1.91%. At a dose of 100 µg/ml LAA showed the lowest level of inhibition (17.02 ± 1.45%). It was identified that the IC50 values for PP, LAA, and PPLAA were 310.71, 322.39, and 305.95 µg/ml respectively (Table 10).

Table 10: Superoxide dismutase radical scavenging potential of PP, LAA and PPLAA

Conc µg/ml	**PP%**	**LAA %**	**PPLAA %**
100	18.89 ± 1.03	17.02 ± 1.45	19.01 ± 1.77
200	34.63 ± 1.77	31.63 ± 2.97	34.78 ± 0.92
300	50.31 ± 1.54	48.31 ± 2.45	52.88 ± 1.20
400	67.52 ± 1.78	62.57 ± 2.87	69.13 ± 1.33
500	80.16 ± 1.91	78.61 ± 1.93	83.41 ± 1.08
IC 50 Values	310.71	322.39	305.95
Values represented mean ± SD of three independent experiments with three replicates each			

DISCUSSION

Antioxidants are vital chemicals that help to protect people by delaying, intercepting, and avoiding oxidative processes triggered by free radicals.(2) Antioxidants are increasingly being employed to balance reactive oxygen species due to their unique capacity. Plant-derived antioxidants are harmless and inhibit the activity of free radicals, protecting the body against a range of ailments. The presence of particular phytochemicals in plant extracts might explain their antioxidant activity.(1) For, antioxidant activity, the PP, LAA, and PPLAA were assessed.

The ability of natural products to donate electrons is tested by bleaching a purple-colored solution with 2,2′-DPPH.(23) In the present work, the concentrations of PP, LAA, and PPLAA scavenged DPPH to varying degrees; with the 500 µg/mL concentration of PPLAA is the suitable scavenger. 100 µg/mL methanolic extracts of S. torvum and S. nigrum fruits and foliage had strong DPPH radicals cavenging activity found by Loganayaki et al., 2010.

According to Bienert et al. (2006), hydrogen peroxide (H2O2) is a ROS that can harm or kill cells.(24) PP, LAA, and PPLAA have demonstrated a noticeable H2O2 scavenging effect. Five concentrations were examined. PPLAA at 500 µg/ml has strong scavenging action on H2O2 (83%). The earlier researcher, Kaur et al. (2006), discovered that the ethanolic extract of Punica granatum possesses efficient scavenging ability on H2O2 molecules at the same time.(25) Enzymatic and non-enzymatic antioxidants work together to effectively scavenge H2O2 by the PP, LAA, and PPLAA.

In the present study, PP, LAA, and PPLAA inhibited the formation of anions in a dose- dependent way. Maharana et al., 2010 also observed in the leaf extract of S. nigrum scavenged (51.7% NO at a concentration of 500 µg/mL).(26)

Membrane function may also be hampered by elevated lipid peroxidation. Lipid peroxidation inhibited PP, LAA, and PPLAA in the current investigation, reducing the toxicity of free radicals and strongly bolstering antioxidant activity. Lipid peroxidation was used to demonstrate the extracts' (Phyllanthus niruri) antioxidant properties.(27)

The ABTS assay was used to measure antioxidant activity.(28) At 500 µg/ml doses, the PP, LAA, and PPLAA showed the highest percentage of inhibition in this investigation. These results were similar to those of Badami et al. (2005) who used pharmaceuticals to detect antioxidant activity.(29)

The most hazardous and reactive free radical is the hydroxyl radical, when measured against other ROS. The hydroxyl radical is considered a harmful species in pathophysiological processes because it can damage almost every molecule in a biological system and contribute to cytotoxicity, carcinogenesis, and mutagenesis (Babu et al., 2001).(30) The medications stopped the breakdown of deoxyribose and scavenged hydroxyl radicals when they were added to the process mixture. Similarly, Arockiamary and Vijayalakshmi (2014) demonstrated that the hydroxyl radical scavenging activity was steadily increased by increasing the concentration of Solanum tuberosm from 100g to 2000g.(31)

The present study found that PP, LAA, and PPLAA inhibit the bleaching process of β-carotene, lowering the toxicity of free radicals and so bolstering its antioxidant properties. The Ramamurthy et al., 2012 study was comparable to the current one.(32)

Another important indicator of hepatocellular injury in both acute and chronic circumstances is succinate dismutase. In order to lessen the damaging effects of this radical, SOD may have a role in scavenging superoxide anion to produce hydrogen peroxide (Chance et al., 1952).(33) Different PP, LAA, and PPLAA doses had dose-dependent SOD activity. Similarly, Gindi et al. (2013) found that the amount of SOD in Solanum spp. methanolic extract.(33)

CONCLUSION

This study highlights the antioxidant potential of PP, LAA, and their combination (PPLAA) through the in vitro methods. These results support the potential application of these compounds in formulations aimed at mitigating oxidative stress and related disorders. Further studies involving in vivo models are recommended to validate these findings.

REFERENCES

1. Armoskaite V, Ramanauskiene K, Maruska A, Razukas A, Dagilyte A, Baranauskas A, et al. The analysis of quality and antioxidant activity of green tea extracts. J Med Plants Res. 2011;5(5):811–6.
2. Velioglu YS, Mazza G, Gao L, Oomah BD. Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. J Agric Food Chem. 1998;46(10):4113–7.
3. Sun W, Sanderson PE, Zheng W. Drug combination therapy increases successful drug repositioning. Drug Discov Today. 2016;21(7):1189–95.
4. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal Biochem. 1999;269(2):337–41.
5. Kasangana PB, Haddad PS, Stevanovic T. Study of polyphenol content and antioxidant capacity of Myrianthus arboreus (Cecropiaceae) root bark extracts. Antioxidants. 2015;4(2):410–26.
6. Aliyu AB, Ibrahim MA, Ibrahim H, Musa AM, Lawal AY, Oshanimi JA, et al. Free radical scavenging and total antioxidant capacity of methanol extract of Ethulia conyzoides growing in Nigeria. Rom Biotechnol Lett. 2012;17(4):7458–65.
7. Phatak RS, Hendre AS. Total antioxidant capacity (TAC) of fresh leaves of Kalanchoe pinnata. J Pharmacogn Phytochem. 2014;2(5):32–5.
8. Yen GC, Chen HY. Antioxidant activity of various tea extracts in relation to their antimutagenicity. J Agric Food Chem. 1995;43(1):27–32. doi:10.1021/jf00049a007
9. Manimala MRA, Murugesan R. In vitro antioxidant and antimicrobial activity of carotenoid pigment extracted from Sporobolomyces sp. isolated from natural source. J Appl Nat Sci. 2014;6(2):649–53.
10. Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis. 1989;10(6):1003–8.
11. Gulcin D. Antioxidant and antiradical activities of L-carnitine. Life Sci. 2006;78(8):803–11.
12. Elmastas M, Gulcin D, Ozturk L, Gokce D. Investigation of antioxidant properties of spearmint (Mentha spicata L.). Asian J Chem. 2005;17:137–48.
13. Boora F, Chirisa E, Mukanganyama S. Evaluation of nitrite radical scavenging properties of selected Zimbabwean plant extracts and their phytoconstituents. J Food Process. 2014;1–7.
14. Adithya ES, Lakshmi MS, Christabel PH, Sasikumar JM. In vitro antioxidant, anti-lipid peroxidation activities and HPLC analysis of methanol extracts from bark and stem of Mahonia leschenaultia Takeda. Asian J Plant Sci Res. 2013;3(2):116–26.
15. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation-decolorizing assay. Free Radic Biol Med. 1999;26(9–10):1231–7.
16. Indu H, Seenivasan R. In vitro antioxidant activity of selected seaweeds from southeast coast of India. Int J Pharm Pharm Sci. 2013;5(2):474–84.
17. Winterbourn CC, Hawkins RE, Brian M, Carrell RW. The estimation of red cell superoxide dismutase activity. J Lab Clin Med. 1975;85(2):337–41.
18. Pandithurai M, Murugesan S. Free radical scavenging activity of methanolic extract of brown alga Spatoglossum asperum. J Chem Pharm Res. 2014;6(7):128–32.
19. Chung SK, Osawa T, Kawakishi S. Hydroxyl radical-scavenging effects of species and scavengers from brown mustard (Brassica nigra). Biosci Biotechnol Biochem. 1997;61(1):118–23.

20. Zargar M, Azizah H, Roheeyati AM, Fathimah AB, Jahanshiri F, Pak-Dek MS. Bioactive compounds and antioxidant activity of different extracts from Vitex negundo leaf. J Med Plants Res. 2011;5(12):2525–32.
21. Kakkar P, Das B, Viswanathan P. A modified spectrophotometric assay of superoxide dismutase. Indian J Biochem Biophys. 1984;21(2):130–2.
22. Nunes PX, Silva SF, Guedes RJ, Almeida S. Biological oxidations and antioxidant activity of natural products. In: Rao V, editor. Phytochemicals as Nutraceuticals – Global Approaches to Their Role in Nutrition and Health. InTech; 2012. CC BY 3.0.
23. Bienert GP, Schjoerring JK, Jahn TP. Membrane transport of hydrogen peroxide. Biochim Biophys Acta. 2006;1758(8):994–1003.
24. Kaur G, Jabbar Z, Athar M, Alam MS. Punica granatum (Pomegranate) flower extract possesses potent antioxidant activity and abrogates Fe-NTA induced hepatotoxicity in mice. Food Chem Toxicol. 2006;44(7):984–93.
25. Maharana L, Pattnaik S, Kar DM, Sahu P, Si SC. Antioxidant activity of aqueous extract of aerial parts of Mollugo pentaphylla L.: an in vitro study. Pharmacologyonline. 2010;3:982–97.
26. Harish R, Shivanandappa T. Antioxidant activity and hepatoprotective potential of Phyllanthus niruri. Food Chem. 2006;95(2):180–5.
27. Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L, Byrne DH. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. J Food Compos Anal. 2006;19(6–7):669–75.
28. Badami S, Prakash OM, Dongre SH, Suresh B. In vitro antioxidant properties of Solanum pseudocapsicum leaf extracts. Indian J Pharmacol. 2005;37(5):251.
29. Babu BH, Shylesh BS, Padikkala J. Antioxidant and hepatoprotective effect of Acanthus ilicifolius. Fitoterapia. 2001;72(3):272–7.
30. Arockiamary, Vijayalakshmi. In vitro antioxidant assay and anticarcinogenic effect of Solanum tuberosum in HEPG2 cell line. Int J Biol Pharm Res. 2014;5(1):78–82.
31. Ramamurthy CH, Kumar MS, Sujatha V. Evaluation of antioxidant, radical scavenging activity and polyphenolic profile in Solanum torvum L. fruits. J Food Sci. 2012;77(9):907–13.
32. Chance B, Greenstein DS, Roughton FJW. The mechanism of catalase action. I. Steady-state analysis. Arch Biochem Biophys. 1952;37(2):301–21.
33. Gindi S, Methra T, Chandu BR, Boyina R, Dasari V. Antiurolithiatic and in vitro antioxidant activity of leaves of Ageratum conyzoides in rat. World J Pharm Pharm Sci. 2013;2:636–49.