Synergistic antioxidant activity of green tea with some herbs

Abstract

Cardiovascular diseases, cancer, arthritis, etc. are caused by free radicals that are byproducts of metabolic pathways. Selected plants namely *Vitis vinifera*, *Phyllanthus emblica* L., *Punica granatum*, *Cinnamomum cassia*, *Ginkgo biloba* L., and *Camellia sinensis* Linn. are reported to produce antioxidant property. This study is undertaken to support the hypothesis that formulation of a polyherbal combination of these plants shows a synergistic effect with green tea. The extracts of each drug were characterized by phytochemical studies and tests for phenolics and flavonoids. *In vitro* antioxidant activity for individual drug and its combination was determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH), superoxide, and nitric oxide free radical scavenging methods. Our results suggest that a combination of all these herbs with green tea can synergistically enhance antioxidant activity and thus lower doses of each herb with green tea may be used. Antioxidant potential of polyherbal combination was also comparable to that of standard ascorbic acid. Studies showed that selected individual plants contained abundant quantity of phenolics and flavonoids and their polyherbal combination with green tea was found to produce best antioxidant activity among all individual extracts. This will help in avoiding undesirable side effects due to higher doses of single herb.

Key words: Antioxidant, DPPH, flavonoids, IC<sub>50</sub>, nitric oxide, phenolics, polyherbal, superoxide radical, synergism

INTRODUCTION

The morbidity and mortality due to various cardiovascular diseases, cancer, and arthritis is alarming. Certain fruits and vegetables containing antioxidants such as ascorbic acid, carotenoids, flavonoids, and hydrolysable tannins play an important role for treating various diseases. Antioxidants are the substances which significantly destroy the free radicals—reactive oxygen species responsible for degenerative diseases. Major plant antioxidants are secondary metabolites of the shikimic acid pathway and phenyl-propanoid metabolism that includes phenolics, coumarins, tannins, chalcone, flavonoid, etc. Flavonoids—flavones, flavanones, flavonols, isoflavones, anthocyanin, chalcone—also inhibit cytotoxic low density lipoproteins (LDL). Tea, the most widely used beverage, is obtained from plant *Camellia sinensis* leaves, family theaceae. The prominent flavonoid in tea is the flavon-3-ols, catechin, epicatechin, epicatechin gallate, epigallocatechin gallate, and their fermentative products—theoflavins, thearubigin. Dry green and black tea leaves have comparable amount of flavonoid and green tea contains most of catechin, while on fermentation catechin decreases but flavones, quercetin, kaempherol, and myricitin are not affected.

Other various plants rich in antioxidant which are taken in this research are *Vitis vinifera* (grape seed), *Gingko biloba*, *Phyllanthus emblica* L. (amla), *Punica granatum* (anar), *Cinnamomum cassia* (dalchini).

*Vitis vinifera* L. (grape seed) are a rich source of monomeric phenolic compounds such as catechin, epicatechin, dimeric,
trimeric, and tetramer proanthocyanidins[7] having antioxidant activity against reactive oxygen species (ROS).[8] The grape seed extract has proved helpful in various diseases such as hepatic fibrosis, ischemia-reperfusion injury (reduces size of infarction in cardiac ischemia), cancer and also inhibits the free radical production.[9,10]

**Punica granatum** L. (anar), belonging to family puniceae, is reported in folklore to be used for several diseases. The peels are a rich source of antioxidants due to high phenolic and tannin contents[11] and peel has much higher antioxidant activity as compared to seeds and other parts.[12] The pomegranate peel extract also gives effective protection against carbon tetrachloride induced hepatotoxicity.[13]

**Phyllanthus emblica** L. (amla) belongs to family euphorbiaceae and rich in ascorbic acid, forms major constituent of many polyherbal formulation described in Charaka samhita.[14] This has revitalizing, anabolic, adaptogenic, anti-stress, immunomodulatory, and memory facilitating effects.[15]

**Cinnamomum cassia** L. is a flavoring ingredient used in food products and has shown usefulness in glucose intolerance and diabetes, antimicrobial activity, treats various cancer cell lines.[16] This plays protective action against lipid peroxidation, quench hydroxyl radical, and hydrogen peroxide.[17]

**Ginkgo biloba** L. tree (Maiden hair tree) belongs to family Ginkgoaceae. Its leaves are beneficial for heart, lungs, inhalation of leaves decoction is effective in bronchial asthma.[18] This is also used for cerebral insufficiency due to degenerative or vascular causes.[19,20] This along with antioxidant activity has several other uses like cyclonucleotide phosphodiesterase inhibition, improvement in cognitive function.[21]

Various in vitro models are used to assess antioxidant activity like DPPH, nitric oxide and superoxide free radical scavenging, etc. Total phenolic and flavonoid contents are also calculated to determine the antioxidant activity.[22,23]

In this study we have selected six plants *Camellia sinesis* (green tea), *Vitis vinifera* (grape seed), *Gingko biloba*, *Phyllanthus emblica* L. (amla), *Punica granatum* (anar), *Cinnamomum cassia* (dalchini) on the basis of their reported uses. This study is undertaken to support the hypothesis that formulation of a polyherbal combination of these plants shows a synergistic effect with green tea. We have determined the antioxidant activity of individual plant and their combination by various in vitro models.

**MATERIALS AND METHODS**

**Drugs and Chemicals**

Chemicals used in all experimental studies are: 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteu (FC) reagent, sodium nitrite, sulfanilamide, sodium nitroprusside, N-1,naphthylethylene-diaminedihydrochloride (NADH), butylated hydroxyl anisole (BHA), phenazine methosulfate (PMS) ascorbic acid, ethylenediamine tetra-acetic acid, phosphoric acid, nitroblue tetrizolium (NBT), ferrous ammonium sulfate, dimethyl sulfoxide (DMSO), hydrogen peroxide, etc. – were obtained from commercial suppliers and used without further purification.

**Plant Materials**

The green tea (*Camellia sinesis* Linn.) harvested from Darjeeling gardens in the month of May by the local gardeners dried at 45°C in oven before extraction. Grape fruits (*Vitis vinifera* Linn.) known as Australian Grapes were purchased as large clusters with red berries from a local market in the month of April, seeds were separated from pulp and dried in air in a shed for 1-2 weeks, further powdered in a grinder. Ripened fruits of anar (*Punica granatum*) were purchased from a local market in the month of November, peels were separated from and cut into pieces and dried in air. Dried pieces were finally powdered for making it ready for extraction. Fresh amla (*Phyllanthus emblica*) fruits were collected in the month of February and air dried in an oven at 40°C and grounded in a cutting mill before extraction. The bark of *Cinnamomum cassia* was purchased after rainy season from the local herbal store. Further, it was dried in shade for 2-3 weeks and grounded before extraction. The *Ginkgo biloba* L. extract was a kind gift from Dalian Hongjiu Biotech Co. Ltd., China.

The identification and authentication were done at Department of Botany, Mata Jeeja Bai Government P. G. Girls College, Indore, Madhya Pradesh, India. The voucher specimens have been preserved in laboratory for future referencing (T-21, V-36, PG-69, E-71, and C-63).

**Extraction**

After selection, each fresh herb was washed properly by tap water followed by distilled water to remove the surface debris. Preparation of the extract was done with different solvents. *Camellia sinesis* (green tea) was extracted with water, *Vitis vinifera* (grape seed) with ethanol (95%) and water, *Phyllanthus emblica* L. fruit (amla) with methanol, *Punica granatum* (anar) with methanol, *Cinnamomum cassia* bark (dalchini) with ethanol (95%) and water. All plant extraction was done in a herb: Solvent ratio 1:10 at 30-80°C. Further these extracts were dried under vacuum and lyophilizer. After that we mixed all extracts in a specific ratio in methanol for polyherbal combination and analysis was carried out.

**Qualitative Phytochemical Analysis**

The preliminary qualitative phytochemical analysis was performed for all six extracts for screening of different chemical groups. All extracts were evaluated further [Table 1].
The capability to scavenge the DPPH radical is calculated using the following equation:

\[ \text{DPPH scavenge} = \frac{A_{\text{cont}} - A_{\text{test}}}{A_{\text{cont}}} \times 100 \]

where \( A_{\text{cont}} \) is the absorbance of control reaction and \( A_{\text{test}} \) is the absorbance in the presence of the sample extract. The antioxidant activity of the extract shall be expressed as IC_{50}. The IC_{50} value is defined as the concentration (mg/ml) of the extract that inhibits the formation of DPPH radicals by 50% [Table 2].

## Nitric Oxide Scavenging Activity

Aqueous solution of sodium nitroprusside at physiological pH spontaneously releases nitric oxide that reacts with oxygen to produce nitrite ions, which can be estimated by the use of Griess reagent. The scavengers of nitric oxide reduce the production of nitrite ions. The reaction mixture (3 ml) containing sodium nitroprusside (10 mM, 2 ml), phosphate buffer saline (0.5 ml), and the individual extract and combination of the standard solution (0.5 ml) was incubated at 25°C for 2.5 h. After incubation, 0.5 ml of the reaction mixture was pipette out mixed with 1 ml of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for the completion of diazotization [Table 3]. 1-Naphthylamine (1 ml, 5%) was added, mixed, and allowed to stand for 0.5 h. A pink colored chromophore was formed; the absorbance of this solution was measured at 540 nm against the corresponding blank solution. Reduction in nitric oxide free radicals was calculated using the formula:

\[ \% \text{Inhibition} = \frac{[\text{Absorbance of blank} - \text{Absorbance of test}] \times 100}{[\text{Absorbance of blank}]} \]

## Superoxide Radical Scavenging Assay

The reaction mixture consisting of 1 ml of NBT solution (156 mM NBT in phosphate buffer, pH 7.4), 1 ml NADH solution (468 mM NADH in phosphate buffer, pH 7.4), and 1 ml of sample solution of the individual extract and combination (50 mg/ml) were reacted with aluminum chloride for the determination of the flavonoid content as described above. Total flavonoid contents were assessed approximately as microgram of gallic acid equivalent by using an equation:

\[ \text{Total Flavonoid Content} = \frac{[\text{Absorbance of blank} - \text{Absorbance of test}] \times 100}{[\text{Absorbance of blank}]} \]

The capability to scavenge the DPPH radical is calculated using the following equation:

\[ \text{DPPH scavenge} = \frac{A_{\text{cont}} - A_{\text{test}}}{A_{\text{cont}}} \times 100 \]

where \( A_{\text{cont}} \) is the absorbance of control reaction and \( A_{\text{test}} \) is the absorbance in the presence of the sample extract. The antioxidant activity of the extract shall be expressed as IC_{50}. The IC_{50} value is defined as the concentration (mg/ml) of the extract that inhibits the formation of DPPH radicals by 50% [Table 2].

## Antioxidant Assay

### DPPH free radical scavenging activity

This is the most widely reported method for screening of antioxidant activity based on the reduction of methanolic solution of colored free radical DPPH – by a free radical scavenger. The procedure involves measurement of decrease in absorbance of DPPH at its absorption maxima of 517 nm. The activity is expressed as inhibitory concentration IC_{50}.

DPPH solution (0.5 mM in methanol) was prepared. Different concentrations of extracts (0.1 ml) and DPPH solution (4.9 ml) were mixed; after 30 min the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity.

### Total Phenol Assay

The total phenolic content was measured by colorimetric assay, using catechin for preparing the calibration curve. The diluted standard solutions (0.5 ml) were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm. Similarly, 1 ml of all individual extracts (200 mg/ml) and combination (50 mg/ml) were reacted with aluminum chloride for the determination of the flavonoid content as described above. Total flavonoid contents were calculated as catechin equivalent by using an equation obtained from the standard curve of gallic acid.

### Total Flavonoid Assay

The total flavonoid content was measured by colorimetric assay, using catechin for preparing the calibration curve. The diluted standard solutions (0.5 ml) were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm. Similarly, 1 ml of all individual extracts (200 mg/ml) and combination (50 mg/ml) were reacted with aluminum chloride for the determination of the flavonoid content as described above. Total flavonoid contents were calculated as catechin equivalent by using an equation obtained from the standard curve of gallic acid.

### Total Phenolic Content

Total phenolic contents were assessed approximately by using the FC phenol reagent using tannic acid as a standard phenolic compound. Phospho molybdic acid in the FC reagent in an alkaline medium produces a blue color complex (molybdnum blue), absorbance was measured at 725 nm using a double beam UV-Visible spectrophotometer (Shimadzu UV-1601, Japan). The total concentration of phenolic compounds in the extract was determined as microgram of gallic acid equivalent by using an equation obtained from the standard curve of gallic acid.

### Table 1: Phytochemical analysis of extracts

| Phytoconstituents | Camellia sinensis (water) | Vitis vinifera (ethanol + water) | Phyllanthus emblica L. (methanolic) | Punica granatum (aqueous) | Cinnamomum cassia (ethanol-water) | Ginkgo biloba L. (methanol) |
|------------------|---------------------------|---------------------------------|-----------------------------------|---------------------------|---------------------------------|-----------------------------|
| Alkaloids        | +                         | –                               | +                                 | +                         | –                               | +                           |
| Saponins         | +                         | +                               | –                                 | +                         | +                               | –                           |
| Tannins          | +                         | –                               | –                                 | –                         | +                               | –                           |
| Flavonoids       | +                         | +                               | +                                 | +                         | +                               | +                           |
| Phenolic compound| +                         | +                               | +                                 | +                         | +                               | +                           |
| Phytosterols     | +                         | +                               | +                                 | –                         | –                               | –                           |
| Terpenoid        | –                         | –                               | –                                 | –                         | +                               | –                           |
| Flavone glycosides| +                        | +                               | +                                 | +                         | –                               | –                           |
| Carbohydrates    | +                         | –                               | +                                 | –                         | +                               | –                           |
| Proteins         | +                         | –                               | +                                 | –                         | –                               | –                           |

Not detected; +: Detected
### Table 2: DPPH radical scavenging activity in % inhibition and IC$_{50}$

| Concentration (mg/ml) | Ascorbic acid | Camellia sinensis | Vitis vinifera | Phyllanthus emblica L. | Punica granatum | Cinnamomum cassia | Ginkgo biloba L. | Combination |
|-----------------------|---------------|-------------------|----------------|-----------------------|----------------|------------------|----------------|------------|
| 10                    | 17.29 ± 1.4    | 10.82 ± 1.2       | 19.92 ± 1.8    | 6.7 ± 1.2             | 4.3 ± 1.2      | 3.8 ± 1.2        | 2.76 ± 1.2     | 27.8 ± 1.2 |
| 20                    | 31.20 ± 2.2    | 12.70 ± 2.4       | 25.7 ± 2.8     | 17.2 ± 2.4            | 8.8 ± 2.4      | 9.5 ± 2.4        | 3.3 ± 2.4      | 34.7 ± 2.4 |
| 30                    | 35.71 ± 2.2    | 13.96 ± 2.4       | 30.45 ± 2.8    | 23.1 ± 2.4            | 12.2 ± 2.4     | 13.7 ± 2.4       | 6.3 ± 2.4      | 42.2 ± 2.4 |
| 40                    | 40.33 ± 2.4    | 17.1 ± 2.4        | 42.6 ± 2.8     | 26.8 ± 2.4            | 16.9 ± 2.4     | 18.9 ± 2.4       | 10.3 ± 2.4     | 46.8 ± 2.4 |
| 50                    | 49.24 ± 2.4    | 21.6 ± 2.8        | 52.8 ± 3.2     | 32.1 ± 3.4            | 22.7 ± 3.4     | 21.9 ± 3.4       | 14.2 ± 3.4     | 52.2 ± 3.4 |
| 60                    | 53.57 ± 3.4    | 38.21 ± 3.4       | 62.5 ± 3.8     | 40.2 ± 3.4            | 25.3 ± 3.4     | 34 ± 3.4         | 17.2 ± 3.4     | 57.7 ± 3.4 |
| 80                    | 58.8 ± 4.2     | 41.16 ± 4.2       | 68.4 ± 4.7     | 47.7 ± 4.7            | 29.9 ± 4.7     | 42.4 ± 4.7       | 26.8 ± 4.7     | 69.3 ± 4.7 |
| 100                   | 65.9 ± 4.2     | 49.6 ± 4.2        | 71.6 ± 4.8     | 51.8 ± 4.8            | 33.1 ± 4.8     | 51.6 ± 4.8       | 29.9 ± 4.8     | 76.8 ± 4.8 |
| 120                   | 70.86 ± 4.2    | 57.8 ± 4.2        | 75.1 ± 4.8     | 58.4 ± 4.8            | 37.5 ± 4.8     | 57.1 ± 4.8       | 35.78 ± 4.8   | 81.0 ± 4.8 |
| 140                   | 77.25 ± 4.2    | 68.7 ± 4.2        | 80.6 ± 4.8     | 63.1 ± 4.8            | 46.8 ± 4.8     | 60.9 ± 4.8       | 41.6 ± 4.8     | 88.17 ± 4.8 |
| 160                   | 83.64 ± 4.2    | 75.78 ± 4.2       | 83.1 ± 4.8     | 73.3 ± 4.8            | 54 ± 4.8       | 65.6 ± 4.8       | 46.2 ± 4.8     | 96.32 ± 4.8 |
| 180                   | 94.22 ± 4.2    | 81.13 ± 4.2       | 83.8 ± 4.8     | 79.8 ± 4.8            | 61 ± 4.8       | 70.6 ± 4.8       | 51.3 ± 4.8     |             |
| 200                   | 94.22 ± 4.2    | 81.13 ± 4.2       | 83.8 ± 4.8     | 79.8 ± 4.8            | 61 ± 4.8       | 70.6 ± 4.8       | 51.3 ± 4.8     |             |
| 220                   | 94.22 ± 4.2    | 81.13 ± 4.2       | 83.8 ± 4.8     | 79.8 ± 4.8            | 61 ± 4.8       | 70.6 ± 4.8       | 51.3 ± 4.8     |             |
| 240                   | 94.22 ± 4.2    | 81.13 ± 4.2       | 83.8 ± 4.8     | 79.8 ± 4.8            | 61 ± 4.8       | 70.6 ± 4.8       | 51.3 ± 4.8     |             |
| 260                   | 94.22 ± 4.2    | 81.13 ± 4.2       | 83.8 ± 4.8     | 79.8 ± 4.8            | 61 ± 4.8       | 70.6 ± 4.8       | 51.3 ± 4.8     |             |
| 280                   | 94.22 ± 4.2    | 81.13 ± 4.2       | 83.8 ± 4.8     | 79.8 ± 4.8            | 61 ± 4.8       | 70.6 ± 4.8       | 51.3 ± 4.8     |             |

% Inhibition = [(A$_0$ - A$_i$)/A$_0$] × 100

where A$_0$ is the absorbance of the control and A$_i$ is the absorbance of the test sample.

### Results and Discussion

After extensive literature search, we have selected five plants for making a polyherbal mixture with the green tea extract to give antioxidant potential in a synergistic manner.[30] The preliminary qualitative phytochemical analysis [Table 1] revealed that all the extracts showed the presence of carbohydrates, proteins, amino acids, steroids, glycosides, flavanoids, tannins, and polyphenols. Results also showed that the total phenolic content calculated on the basis of gallic acid reference to a standard curve ($y = 0.008 x - 0.004$, $r^2 = 0.998$) for Camellia sinensis, Vitis vinifera, Phyllanthus emblica L., Punica granatum, Cinnamomum cassia, Ginkgo biloba L. and polyherbal combination of tea and individual extracts in the ratio of (5:3:3:3:3:3) was found to be 580, 680, 418, 400, 380, 240, 2500 mg of gallic acid eqv./10g, respectively. Total flavonoid contents calculated from the catechin standard curve ($y = 0.011 x - 0.004$, $r^2 = 0.998$) was found to be 410, 450, 160, 120, 170, 80, and 1237 mg of
Table 3: Nitric oxide scavenging in % inhibition and IC50

| Concentration (mg/ml) | Ascorbic acid | Camellia sinensis | Vitis vinifera | Phyllanthus emblica L. | Punica granatum | Cinnamomum cassia | Ginkgo biloba L. | Combination |
|-----------------------|---------------|-------------------|----------------|------------------------|----------------|------------------|----------------|-------------|
| 10                    | 38.1 ± 1.2    | 14.8 ± 2.3***     | 22.05 ± 1.1***| 13.46 ± 2.3***         | 7.0 ± 1.0***   | 3.15 ± 1.0***    | 7.3 ± 1.0***   | 30.12 ± 1.2*** |
| 20                    | 43.4 ± 2.3*** | 18.5 ± 1.0***     | 27.53 ± 1.1***| 24.15 ± 2.1***         | 19.2 ± 2.4***  | 9.40 ± 2.4***    | 10.6 ± 2.4***  | 37.98 ± 2.4*** |
| 40                    | 45.8 ± 2.3*** | 24.24 ± 1.2***    | 35.36 ± 0.86***| 33.78 ± 2.4***         | 19.2 ± 2.4***  | 21.04 ± 2.4***   | 14.97 ± 2.4*** | 50.78 ± 2.4*** |
| 60                    | 57.4 ± 1.3*** | 41.03 ± 1.3***    | 44.57 ± 2.6***| 43.36 ± 2.6***         | 26.2 ± 2.4***  | 28.04 ± 2.4***   | 21.5 ± 2.4***  | 59.19 ± 2.4*** |
| 80                    | 64.79 ± 3.4***| 51.66 ± 1.8***    | 54.12 ± 0.8***| 52.69 ± 1.8***         | 30.29 ± 2.6*** | 32.37 ± 2.6***   | 28.85 ± 2.6*** | 71.79 ± 2.6*** |
| 100                   | 67.25 ± 4.5   | 55.86 ± 1.0***    | 66.19 ± 1.6   | 58.82 ± 1.6           | 43.78 ± 2.8*** | 57.61 ± 2.8***   | 32 ± 1.2***    | 77.95 ± 1.2*** |
| 120                   | 74.95 ± 1.5   | 59.5 ± 1.4***     | 70.05 ± 1.5   | 66.35 ± 1.5           | 52.18 ± 2.5*** | 61.29 ± 2.5***   | 37.3 ± 1.5***  | 81.02 ± 1.5*** |
| 140                   | 81.88 ± 3.4   | 62.6 ± 1.2***     | 72.48 ± 1.2   | 71.80 ± 1.2           | 59.0 ± 1.4***  | 67.25 ± 1.4***   | 46.7 ± 1.4***  | 89.96 ± 1.4*** |
| 160                   | 87.12 ± 2.7   | 65.32 ± 1.2***    | 75.8 ± 1.5   | 76.15 ± 1.5           | 65.4 ± 1.5***  | 71.10 ± 1.5***   | 57.30 ± 1.5*** | 91.58 ± 1.5*** |
| 180                   | 92.5 ± 1.2*** | 79.49 ± 1.6***    | 79.33 ± 1.1***| 73.9 ± 1.1***         | 69.0 ± 2.4***  | 72.5 ± 2.4***    | 68.32 ± 2.4*** | -            |
| 200                   | 97.0 ± 1.6*** | 80.65 ± 2.5***    | 80.65 ± 2.2***| 73.02 ± 2.2           | 73.20 ± 2.4*** | 69.95 ± 2.4***   | -              | -            |
| 220                   | 100.0 ± 1.7   | 81.34 ± 1.5***    | 81.34 ± 1.4** | 73.02 ± 1.4           | 73.20 ± 2.2*** | 69.95 ± 2.2***   | -              | -            |
| IC50 25.6              | 68.67 ± 2.2** | 66.07 ± 1.2**     | 67.14 ± 1.3** | 93.95 ± 1.3**         | 83.68 ± 1.3**  | 108.2 ± 1.3**    | 31.47 ± 1.3**  |             |

Note: Test not performed because activity not increasing significantly further. (***P < 0.001, **P < 0.01, *P < 0.05, ns: Not significant); All values represent the mean ± SD (n = 3).

gallic acid eqv./10g catechin equivalents/g, respectively.

On the basis of preliminary studies conducted using different combinations of 2, 3, 4, 5, and 6 extracts in a different concentration/ratio the best selected ratio of combination which possesses significant synergistic activity was 5:3:3:3:3:3. Synergistically these herbs potentiate green tea to provide a bioactive mechanism to reduce free radical-induced oxidative stress.[31] Recent research[32,33] also shows that through overlapping or complementary effects, the complex mixture of phytochemicals in selected herbs provide a better protective effect on health than single phytoconstituent.

The result showed that combination of all extract in ratio (5:3:3:3:3:3) possessed the highest antioxidant activity (IC50 = 33.5, 31.47, 46.34) among all other combination as well as for individual combination (grape seed IC50 = 44.46, 66.07, 51.05; green tea IC50 = 90.86, 68.67, 59.80; amla IC50 = 97.72, 67.1, 69.82; anar IC50 = 147.6, 93.95, 121.1; cinnamon IC50 = 125.6, 83.68, 91.2; ginkgo IC50 = 134, 108.2, 128.2). Results also revealed that antioxidant potentials of individual extracts and combination were comparable to that of standard ascorbic acid (IC50 = 51.05, 25.6, 48.32).

In summary our results showed that selected individual plants contained abundant quantity of phenolics and flavonoids and the polyherbal combination of all five extracts with green tea was found to produce best antioxidant activity among all individual extracts.

**CONCLUSION**

On the basis of above results, it can be concluded that the antioxidant potential of the plant extract depends on the presence of phenolic compounds and flavonoids. From the past research, it was already proved that the phenolic content is directly responsible for reduction of oxidative stress. The phenolic content and flavonoid content were checked by the FC method and aluminum chloride assay, respectively, and found to be profound in all the plant parts under investigation. The in vitro antioxidant activity was tested by DPPH, nitric oxide and super oxide free radical scavenging activity studies for individual plant extract and combined formulation 5:3:3:3:3:3 of plants Camellia sinensis, Vitis vinifera, Phyllanthus emblica L., Punica granatum, Cinnamomum cassia, and Ginkgo biloba L. Thus, it can be concluded that the selected combination of extracts produce their effect in the synergistic manner with green tea. There is however a scope for confirming the results of this study on relevant animal models followed by studies for clinical support.
in future for development of polyherbal formulation of green tea with respective plant extracts.

REFERENCES

1. Cao G, Mucciutelli HU, Sanchez-Moreno C, Prior RL. Anthocyanins are absorbed in glycated forms in elderly women: A radical prevention capacity using fluorescein as the probe. Am J Clin Nutr 2001;73:920-6.

2. Harborne JB. Phytochemical Methods. 2nd ed. London New York: Chapman and Hall; 1998. p. 209-47.

3. Sharma US, Kumar A. In vitro antioxidant activity of Rubus ellipticus fruits. J Adv Pharm Res 2010;3:175-8.

4. Vadalia DN, Pethi AM, Chakraborty GS. Antioxidant activity of polyherbal formulation, J Pharm Res 2010;3:1756-8.

5. Pal DK, Mitra S. Preliminary study on the in vitro antioxidant activity of the stems of Opuntia vulgaris, J Adv Pharm Tech Res 2010;1:268-72.

6. Michalowska AG, Regula J. Use of tea extracts (Camellia sinensis) in jelly candies as polyphenols sources in human diet. Asia Pac J Clin Nutr 2007;16:43-6.

7. Teresa EB, Yolanda GF, Rivas-Gonzalo JC, Santos-Buelga C. Characterization of proanthocyanidins of Vitis vinifera variety Tinta del Paris grape seeds. J Agric Food Chem 1992;40:1794-9.

8. Bagchi D, Garg A, Krohn RL, Bagchi M, Tran MX, Stohs SJ. Oxygen free radical scavenging abilities of vitamins C and E, and a grape seed proanthocyanidin extract in vitro. Res Commun Mol Pathol Pharmacol 1997;95:179-89.

9. Şehirli O, Özelt Y, Dulundu E, Topaloglu U, Erçan F, Şener G. Grape seed extract treatment reduces hepatic ischemia-reperfusion injury in rats. Phytother Res 2008;22:43-8.

10. Shao ZH, Becker LB, Vanden Hoek TL, Schumacker PT, Li CY, Zhao D, et al. Grape seed proanthocyanidin extract attenuates oxidant injury in cardiomyocytes. Pharmacol Res 2003;47:463-9.

11. Guo CJ, Yang JJ, Wei JY, Li FY, Xu J, Jiang YG. Antioxidant activities of peel, pulp and seed fractions of common fruits as determined by FRAP assay. Nutr Res 2003;23:1719-26.

12. Singh RP, Murthy KN, Jayaprakasha GK. Studies on the antioxidant activity of pomegranate peel and seed extracts using in vitro models. J Agric Food Chem 2002;50:4791-5.

13. Murthy KN, Jayaprakasha GK, Singh RP. Studies on antioxidant activity of pomegranate peel extract using in vitro models. J Agric Food Chem 2002;50:81-8.

14. Shao ZH, Becker LB, Vanden Hoek TL, Schumacker PT, Li CY, Zhao D, et al. Grape seed proanthocyanidin extract attenuates oxidant injury in cardiomyocytes. Pharmacol Res 2003;47:463-9.

15. Sharma PV, Charaka Samhita, Chapter 1Chikitsasthana, New Delhi; Narosa Publishing House; 1997 p. 163.

16. Anderson RA, Broadhurst CL. Isolation and characterization of polyphenol type-A polymers from cinnamon with insulin-like biological activity. J Agric Food Chem 2004;52:65-70.

17. Murcia MA, Egea I, Romozano F, Parras P, Jimenez AM, Martinez-Tome M. Antioxidant evaluation in dessert spices. J Agric Food Nutr 2004;52:1872-81.

Table 4: Superoxide free radical scavenging in % inhibition and IC<sub>50</sub>

| Concentration (mg/ml) | Ascorbic acid | Camellia sinensis | Vitis vinifera | Phyllanthus emblica L. | Punica granatum | Cinnamom cassia | Ginkgo biloba | Combination |
|-----------------------|---------------|-------------------|---------------|-----------------------|----------------|----------------|--------------|-------------|
| 10                    | 19.1 ±        | 9.9 ±             | 22.5 ±        | 12.1 ±                | 3.86 ±         | 5.0 ±          | 1.6 ±       | 11.78 ±     |
| 20                    | 1.2           | 1.4***            | 2.2***        | 1.7***                | 1.8***         | 1.1***         | 1.3***      | 2.3***      |
| 30                    | 28.07 ±       | 14.43 ±           | 29.8 ±        | 19.7 ±                | 6.09 ±         | 12.39 ±        | 3.0 ±       | 19.30 ±     |
| 40                    | 1.3           | 3.4***            | 3.4***        | 1.8***                | 1.2***         | 2.4***         | 2.4***      | 1.2***      |
| 50                    | 35.1 ±        | 20.3 ±            | 36.8 ±        | 22.3 ±                | 10.36 ±        | 20.9 ±         | 4.2 ±       | 25.8 ±      |
| 60                    | 1.2           | 2.3***            | 1.6***        | 1.7**                 | 1.2**          | 1.7**          | 1.1***      | 1.7**       |
| 80                    | 42.88 ±       | 30.28 ±           | 43.29 ±       | 38.6 ±                | 22.76 ±        | 29.2 ±         | 6.9 ±       | 40.8 ±      |
| 100                   | 5.44 ±        | 37.30 ±           | 49.18 ±       | 44.7 ±                | 33.94 ±        | 38.82 ±        | 1.4 ±       | 53.04 ±     |
| 120                   | 2.3           | 1.1***            | 3.4***        | 1.8***                | 3.4***         | 1.2**          | 1.1**       | 2.3***      |
| 140                   | 61.99 ±       | 45.70 ±           | 52.26 ±       | 49.97 ±               | 38.4 ±         | 46.13 ±        | 18.49 ±     | 59.04 ±     |
| 160                   | 2.5           | 2.8**             | 2.1***        | 1.7***                | 6.2**          | 2.2***         | 1.7**       | 2.2**ns     |
| 180                   | 68.19 ±       | 51.43 ±           | 59.1 ±        | 57.2 ±                | 44.7 ±         | 53.65 ±        | 25.81 ±     | 62.8 ±      |
| 200                   | 2.3           | 3.4***            | 1.2**         | 2.2**                 | 1.8**          | 4.6**          | 1.2**       | 1.7**       |
| 220                   | 75.40 ±       | 57 ±              | 62.6 ±        | 64.9 ±                | 46.9 ±         | 59.75 ±        | 33.73 ±     | 81.2 ±      |
| 240                   | 1.8           | 1.1 ns            | 1.2***        | 2.2”                  | 2.2**          | 1.8**          | 4.6**       | 1.2**       |
| 260                   | 84.55 ±       | 67.12 ±           | 65.24 ±       | 70.9 ±                | 50.8 ±         | 65.04 ±        | 44.3 ±      | 90.26 ±     |
| 280                   | 1.8           | 1.1***            | 2.2***        | 2.8***                | 3.1”           | 1.7**          | 3.2**       | 2.4***      |
| 300                   | 33.94 ±       | 72.76 ±           | 68.9 ±        | 74.2 ±                | 57.1 ±         | 70.9 ±         | 53.86 ±     | –          |
| 320                   | –             | 1.2***            | 1.7”          | 3.4***                | 1.7            | 2.2**          | 6.2***      | –          |
| 340                   | –             | 75.4 ±            | 73.1 ±        | 76.5 ±                | 64.0 ±         | 75.43 ±        | 64.6 ±      | –          |
| 360                   | –             | 2.2***            | 1.7**         | 4.2**                 | 1.5**          | 3.2**          | 2.2**”      | –          |
| 380                   | –             | 81.2 ±            | 84.2 ±        | –                     | 65.6 ±         | –              | 68.2 ±      | –          |
| 400                   | –             | 2.4***            | 1.8***        | –                     | 1.2**          | 2.3**          | –          | –          |
| 420                   | –             | –                 | –             | –                     | 72.96 ±        | –              | 71.23 ±     | –          |
| 440                   | –             | –                 | –             | –                     | 2.1**          | 4.2**          | –          | –          |
| IC 50                 | 48.32         | 59.80             | 51.05         | 69.82                 | 121.1          | 91.2           | 128.2       | 46.34       |

<sup>*</sup> Test not performed because activity not increasing significantly further. **P < 0.001, *P < 0.01, *P < .05, ns: Not significant. All values represent the mean ± SD (n = 3).
18. Kleijnen J, Knipschild P. Ginkgo biloba. Lancet 1992;340:1136-9.
19. Pietri S, Maurelli E, Drieu K, Culcasi M. Cardioprotective and antioxidant effects of the terpenoid constituents of Ginkgo biloba extract (EGb761). J Mol Cell Cardiol 1997;29:733-42.
20. Pincemail J, Dupuis M, Nasr C, Hans P, Haag-Berrurier M, Anton R, et al. Superoxide anion scavenging effect and superoxide dismutase activity of Ginkgo biloba extract. Experientia 1989;45:708-12.
21. Clostre F. From the body to cell membrane: The different level of pharmacological action of Ginkgo biloba extract. Presse Med 1986;15:1529-38.
22. Yang WJ, Li DP, Li JK, Li MH, Chen YL, Zhang PZ. Synergistic antioxidant activities of eight traditional Chinese herb pairs, Biol Pharm Bull 2009;32:1021-6.
23. Andreja RI, Ner Hra, Majda H, Eljko K, Davorin B. Comparison of antioxidative and synergistic effects of rosemary extract with α-tocopherol, ascorbyl palmitate and citric acid in sunflower oil. Food Chem. 2000;71:229-33.
24. Singleton VL, Orthofer RM. Analysis of total phenol and other oxidative substrate and antioxidants by means of Folin-Ciocalteu Reagent methods. Enzymol 1999;299:155-7.
25. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total Flavonoid content in propolis by two complementary colorimetric methods, J Food Drug Anal 2002;10:178-82.
26. Shivprasad HN, Mohan H, Kharya MD, Shiradkar M, Lakshman K. In vitro model for antioxidant activity evaluation: A review. Pharmainfo Net 2005;3:1-11.
27. Blois MS. Antioxidant determination by the use of stable free radical. Nature 1958;181:1199-00.
28. Jagetia GC, Rao SK, Baliga MS, Babu KS. The evaluation of nitric oxide scavenging activity of certain herbal formulations in vitro: A preliminary study, Phytother Res 2004;18:561-5.
29. Gülçin I, Alici HA, Cesur M. Determination of in vitro antioxidant and radical scavenging activities of propofol. Chem Pharm Bull (Tokyo) 2005;53:281-5.
30. Guimarães R, Barros L, Carvalho AM, Ferreira IC. Infusions and decoctions of mixed herbs used in folk medicine: Synergism in antioxidant potential. Phytother Res 2011;25:1209-14.
31. Jamuna KS, Ramesh CK, Srinivasa TR, Raghu KL. In-vitro antioxidant studies in some common fruits. Int J Pharm Pharm Sci 2011;3:60-3.
32. Eberhardt MV, Lee CY, Liu RH. Nutritions Antioxidant activity of fresh apples. Nature 2000;405:903-4.
33. Rapola JM, Virtamo J, Ripatti S, Huttunen JK, Albanes D, Taylor PR, et al. Randomised trial of R-tocopherol and β-carotene supplements on incidence of major coronary events in men with previous myocardial infarction. Lancet 1997;349:1715-20.

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