academicJournals

Vol. 7(39), pp. 2898-2905, 17 October, 2013
DOI 10.5897/JMPR2013. 5124
ISSN 1996-0875 © 2013 Academic Journals
http://www.academicjournals.org/JMPR

Journal of Medicinal Plant Research

Full Length Research Paper

Antioxidant and free radical scavenging activity of triphala determined by using different *in vitro* models

Deepa Babu, Prema Gurumurthy*, Sai Krishna Borra and K. M. Cherian

Frontier Lifeline Hospital & Dr. K. M. Cherian Heart Foundation, RC-30, Ambattur Estate road, Mogappair, Chennai, 600101, India.

Accepted 24 September, 2013

The use of antioxidants in treatment of oxidative stress-related pathologies is a possible therapeutical strategy for the future. Natural product with antioxidant properties could trigger this goal. The aim of this *in vitro* study was to assess the antioxidant activity of the ethanolic extract of "Triphala" (an Indian Ayurvedic formulation). The present study describes the effects by di(phenyl)-(2,4,6-trinitrophenyl) iminoazanium (DPPH) scavenging of ferric ion, O_2^-, NO, H_2O_2 models. The ethanolic extracts showed good free radical scavenging property which was calculated as IC_{50}. IC_{50} of ethanolic extracts was found to be 5.94 µg ml^{-1} for DPPH, 32.59 µg ml^{-1} for NO, 16.63 µg ml^{-1} for H_2O_2, 42.95 µg ml^{-1} for O_2^- ,1030 ± 18.54 µM Fe (II)/g for ferric reducing antioxidant power (FRAP), which were compared with ascorbic acid (DPPH, NO, O_2^-, H_2O_2, FRAP). Total antioxidant capacity and total phenol content of the extract was found to be 388.9 µg ml^{-1} ascorbic acid and 254 µg ml^{-1} gallic acid for ethanolic extract. Triphala ethanolic extract exhibited potent free radical scavenging activity. The overall antioxidant activity is attributed to its polyphenolic and other phytochemical constituents. The findings suggest that "Triphala" could be a potential source of natural antioxidant in preventing or slowing the progression of aging and age-associated oxidative stress-related degenerative diseases.

Key words: Triphala, free radicals scavenge, anti-oxidant, di(phenyl)-(2,4,6-trinitrophenyl)iminoazanium (DPPH), ferric reducing antioxidant power (FRAP).

INTRODUCTION

Oxidative stress is linked to inflammation, playing together an important role in the pathogenesis of cancer (Maeda and Omata, 2008), cardiovascular diseases (Montecucco et al., 2011), type 2 diabetes and obesity (Sell and Eckel, 2009). Oxidative stress is an imbalance between production of reactive oxygen species and antioxidant defences (Betteridge, 2000). The redox stress triggers the activation of immune cells which release pro-inflammatory cytokines, reactive oxygen and nitrogen species causing damage to biological molecules and inducing imbalances in physiological and pathological pathways (Lonkar and Dedon, 2011). Epidemiological and *in vivo* studies have provided evidence that dietary intake of antioxidant and anti-inflammatory compounds is a key strategy for health promotion by lowering oxidative stress and inflammation (Watz, 2008).

Currently, synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tert-butylhydroquinone (TBHQ) are widely used in the food industry. However, restriction on the synthetic

*Corresponding author. E-mail: prema.fll@gmail.com. Tel: +91 - 9940116682, +91 – 9952967744.

antioxidants is being imposed because of their toxicity to liver and carcinogenicity (Grice, 1986; Wichi, 1988). Therefore, the development and utilization of more effective antioxidants of natural origins are desired. Of various kinds of natural antioxidants, phenolic compounds have received much attention (Espin et al., 2000; Luo et al., 2002). Therefore, the phenolic constituents of plants are of interest as potential chemo preventive agents, and plants may be an attractive alternative to currently available commercial antioxidants because they are biodegradable to non-toxic products (Lim et al., 2002; Kayano et al., 2002).

Triphala ['three' (tri) 'fruits' (phala)] is a traditional Ayurvedic herbal formulation consisting of the dried fruits of three medicinal plants, *Terminalia chebula, Terminalia bellirica*, and *Phyllanthus emblica*, also known as the 'three myrobalans'. This formulation, rich in antioxidants, is a frequently used ayurvedic medicine to treat many diseases such as anemia, jaundice, constipation, asthma, fever and chronic ulcers. It is an important medicine of the 'rasayana' group and is believed to promote health, immunity and longevity. It corrects constipation, cleanses and tonifies the gastrointestinal tract and also detoxifies the whole body, and improves digestion and assimilation (Nadkarni, 1976). It exhibits anti-viral, anti-bacterial, anti-fungal and anti-allergic properties (Mehta et al., 1999; Singh, 2003). "Triphala" and its constituents act as cardio-tonic, control blood pressure, improve blood circulation and reduce cholesterol levels (Thakur et al., 1988; Tariq et al., 1977). "Triphala" shows immunomodulatory properties and helps in improving the body's defence system (Srikumar et al., 2005).

In recent years, there are also several reports in the literature which suggest that "Triphala" possesses antimutagentic and radioprotecting activity (Kaur et al., 2005, 2002; Vani et al., 1997; Jagetia et al., 2002; Jagetia et al., 2004a, b; Arora et al., 2003; Kumar et al., 1996; Naik et al., 2005). Triphala has been reported to be a rich source of Vitamin C, ellagic acid, gallic acid, chebulinic acid, bellericanin, β-sitosterol and flavanoids (Jagetia et al., 2002).

The use of antioxidants in treatment of oxidative stress-related pathologies is a possible therapeutical strategy for the future. Natural product with antioxidant properties could trigger this goal. The aim of this *in vitro* study was to assess the antioxidant activity of the ethanolic extract of "Triphala".

METHODS AND MATERIALS

Chemicals and reagents

The standard L-ascorbic acid was obtained from Hi-Media lab. Ltd., Mumbai, India. 1,1-diphenyl-2-picryl hydrazyl (DPPH), nitro blue tetrazolium (NADH), phenazine metho sulphate, 2,2'-azobis(2-methypropionamidine) dihydro chloride (AAPH), 2,4,6-tripyridyl-s-triazine gallic acid and folin-ciocalteu reagent were purchased from Sigma Chemical Co, St. Louis, MO, USA. All other chemicals and reagents, all of analytical grade, were from E. Merck (Darmstadt, Germany), unless stated otherwise.

Extraction

T. chebula, T. bellirica and *P. emblica* were bought from the local market and identified by botanist in Captain Srinivasa Murti Drug Research Institute of Ayurveda (CSMDRIA), Chennai. The fruits were dried and pulps were coarsely powdered. 1 kg powder was soaked in ethanol for 7 days with intermittent shaking and the solvent was filtered with Whatman filter paper. The filtrate was evaporated under vacuum drier and the brown mass residue obtained was stored at -4°C for further use(s).

In vitro antioxidant assays

Antioxidant ability assays

The antioxidant activity of the extracts was evaluated by the phosphomolybdenum method according to the procedure of Prieto et al. (1999). Briefly, 0.3 ml of plant extract was combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing reaction solution were incubated at 95°C for 90 min. Then the absorbance of the solution was measured at 695 nm using spectrometer against blank after cooling to room temperature. Ascorbic acid was used as reference standard. The antioxidant activity is expressed as the number of equivalents of ascorbic acid (AscAE).

Total phenol content

The total phenolic content was determined using spectrophotometric method (Singleton et al., 1999). The reaction mixture was prepared by mixing 0.5 ml of ethanolic solution (1 mg/ml) of extract, 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml 7.5% $NaHCO_3$. The samples were incubated at 45°C for 15 min. The absorbance was determined at λ_{max} = 765 nm. The samples were prepared in triplicate and the mean value of absorbance was obtained. Blank was concomitantly prepared with ethanol instead of extract solution. The same procedure was repeated for the gallic acid and the calibration line was constructed. The total phenolic content was expressed in terms of gallic acid equivalent (mg of GaA/g of extract).

DPPH free radical scavenging assay

The free radical scavenging activity of the extract was measured using DPPH by the method of Blios (1958). A 0.1 mM solution of DPPH in methanol was prepared and 1 ml of this solution was added to 3 ml of various concentrations (0.2 to 1.0 mg/ml) of sample dissolved in methanol to be tested. After 30 min, absorbance was measured at 517 nm. Ascorbic acid was used as a reference material. All tests were performed in triplicate. The scavenging activity was calculated as follows:

DPPH radical scavenging activity (%) = [(Absorbance of control – Absorbance of sample)/Absorbance of control] × 100

Reducing power

The reducing power of the extract was determined according to the method of Oyaizu (1986). Briefly, 1.0 ml of different concentration sample (0.2 to 1.0 mg/ml) was mixed with 2.5 ml of a 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of a 1% (w/v) solution of potassium ferricyanide. The mixture was incubated in a water bath at 50°C for 20 min. Afterwards, 2.5 ml of a 10% (w/v) trichloroacetic acid solution was added and the mixture was then centrifuged at 3000 rpm for 10 min. A 2.5 ml aliquot of the upper layer was combined with 2.5 ml of distilled water and 0.5 ml of a 0.1% (w/v) solution of ferric chloride, and absorbance was measured at 700 nm. All tests were performed in triplicate and the graph was plotted with the average of the three determinations.

Ferric reducing antioxidant power (FRAP) assay

The determination of the total antioxidant activity (FRAP assay) in the extract is a modified method of Benzie and Strain (1996). The stock solutions included 300 mM acetate buffer (3.1 g $C_2H_3NaO_2 \cdot 3H_2O$ and 16 ml $C_2H_4O_2$), pH 3.6, 10 mM 2, 4, 6-tripyridyl-s-triazine (TPTZ) solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ, and 2.5 ml $FeCl_3 \cdot 6H_2O$. The temperature of the solution was raised to 37°C before use. 'Triphala' extract (200 µl) was allowed to react with 2800 µl of the FRAP solution for 30 min in the dark condition. Readings of the colored product (ferrous tripyridyltriazine complex) were taken at 593 nm. The standard curve was linear between 200 and 1000 µM $FeSO_4$. Results are expressed in µM Fe (II)/g dry mass and compared with ascorbic acid.

Superoxide anion radical scavenging activity

Measurement of superoxide anion radicals scavenging activity of "Triphala" extract was based on the method described by Nishimiki et al. (1972). To 1 ml of nitroblue tetrazolium (NBT) solution (156 µM NBT in 100 mM phosphate buffer, pH 7.4), 1 ml NADH solution (468 µM in 100 mM phosphate buffer, pH 7.4) and 1 ml of extract (10 to 50 µg/ml) in ethanol was mixed. The reaction started by adding 100 µl of phenazine methosulphate (PMS) solution (60 µM PMS in 100 mM phosphate buffer, pH 7.4) to the mixture. The reaction mixture was incubated at 25°C for 5 min and the absorbance at 560 nm was measured against blank sample. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity.

Hydrogen peroxide scavenging assay

Hydrogen peroxide scavenging potential of the plant extract was determined using the method described by Jayaprakasha et al. (2004). A solution of hydrogen peroxide (20 mM) was prepared in phosphate buffer saline (PBS, pH 7.4). Different concentrations of the extract (20 to 100 µg/ml) in ethanol (1 ml) were added to 2 ml of hydrogen peroxide solution in PBS. After 10 min the absorbance was measured at 230 nm against a blank solution that contained hydrogen peroxide solution without the extract. The percentage of H_2O_2 scavenging of the plant extract was calculated as follows:

% Scavenged $[H_2O_2]$ = [(Abs control – Abs sample) / Abs control] × 100

Nitric oxide radical scavenging assay

The method of Garrat (1964) was used to determine the nitric oxide radical scavenging activity of "Triphala". A volume of 2 ml of 10 mM sodium nitroprusside prepared in phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of "Triphala" at various concentrations ranging from 10 to 50 µg/ml and ascorbic acid at various concentrations ranging from 25 to 200 µg/ml. The mixture was incubated at 25°C. After 150 min, 0.5 ml of incubation solution was withdrawn and mixed with 0.5 ml of Griess reagent [1.0 ml sulfanilic acid reagent (0.33% prepared in 20% glacial acetic acid at room temperature for 5 min with 1 ml of naphthylethylene diamine dihydrochloride (0.1% w/v)]. The mixture was incubated at room temperature for 30 min, followed by the measurement of absorbance at 540 nm using spectrophotometer (Shimadzu UV-1800).

Statistical analysis

Data were expressed as mean ± standard deviation (SD) for three parallel measurements using Graph Pad Prism version 6.0 for windows, Graph Pad Software, San Diego, California, USA. Statistical analysis was done by student's *t*-test and $p < 0.05$ considered as significant. The 50% inhibitory concentration (IC_{50}) was calculated from the dose response curve (Graph Pad Prism Version 6.0) obtained by plotting percentage inhibition versus concentrations.

Percentage (%) inhibition = Absorbance of control – absorbance of test sample/ absorbance of control × 100.

RESULTS AND DISCUSSION

Total antioxidant capacity

The total antioxidant activity of the "Triphala" extract was evaluated by the phosphomolybdenum method and followed by comparing with the standard solution of ascorbic acid equivalents. The standard curve of ascorbic acid was done by using ascorbic acid concentration ranging from 20 to 100 µg/ml. The following equation expressed the absorbance of ascorbic acid standard solution as a function of concentration:

$y = 0.1532x + 0.1296$, $R^2 = 0.9507$

Where x is the absorbance and Y is the ascorbic acid equivalent (mg/g). Ethanolic extract of "Triphala" showed an increase in antioxidant capacity with an increase in dose. Total antioxidant capacity of "Triphala" extract was found to be 388.9 ± 2.54 µg ascorbic acid equivalents at 100 µg/ml "Triphala" extract concentration (Table 2). The phosphormolybdenum method is an important antioxidant assay based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of green phosphate/Mo (V) complex with a maximal absorption at 695 nm and antioxidant capacity of triphala due to its polyphenol content, as polyphenols plays an important

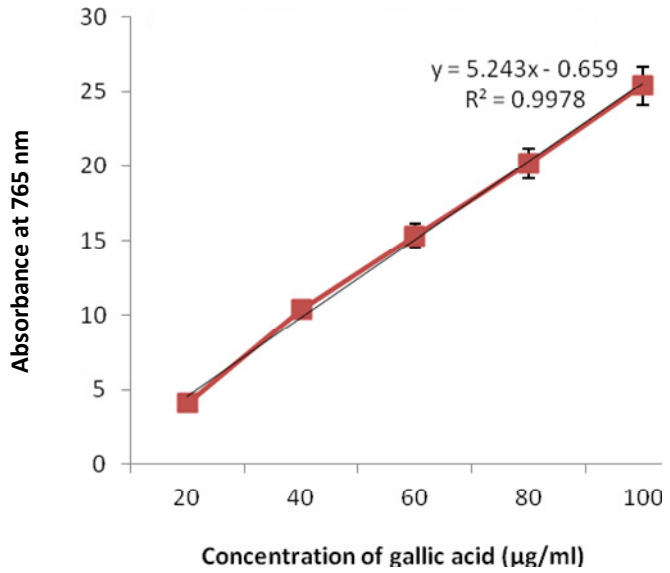

Figure 1. Total phenolic content of Triphala expressed in gallic acid equivalents (mg GAE/g).

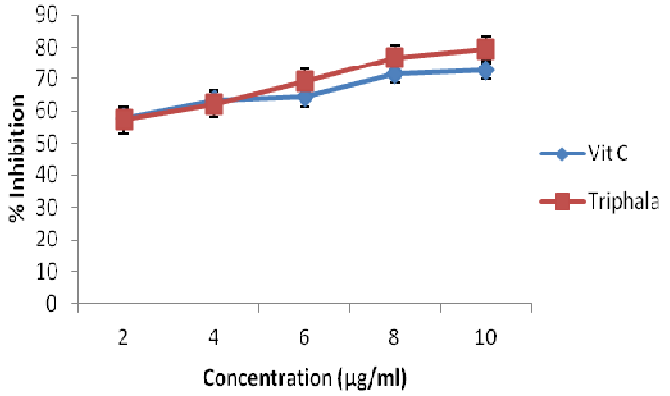

Figure 2. DPPH radical scavenging activity of Triphala and Vitamin C. Data are presented as the percentage of DPPH radical scavenging, Mean± SD

role as antioxidants in living systems due to the presence of hydroxyl groups in *ortho*- and *para* positions.

Total phenolic content

Phenolic antioxidants are potent free radical terminator (Shahidi and Wanasundara, 1992). The phenolic compounds, biologically active component are the main agents that can donate hydrogen to free radicals and thus break the chain reaction of lipid peroxidation at the first initiation step. This high potential of phenolic compounds to scavenge radical may be explained by their phenolic hydroxyl groups (Sawa et al., 1999). In the estimation of total phenolic content, absorbance (at 725 nm) of gallic acid and ethanolic extract of "Triphala" in different concentrations (mg/g) was linear. The phenolic content was expressed as gallic acid equivalents (GAE) detected in "Triphala" extract (100 mg) and yield obtained was 25.4 ± 0.004 mg/g gallic acid-equivalent ($y = 5.243x - 0.659$, $r^2 = 0.9978$) (Figure 1 and Table 2).

DPPH radical scavenging activity

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule which is widely used to investigate radical scavenging activity. In DPPH radical scavenging assay, antioxidants react with DPPH (deep violet color) and convert it to yellow coloured α,α-diphenyl-β-picryl hydrazine. The degree of discoloration indicates the radical-scavenging potential of the antioxidant (Blois, 1958; Huang et al., 2005). The extract was capable of neutralizing the DPPH free radicals via hydrogen donating activity by 16.46, 29.27, 52.10, 70.24, and 84.35% at concentrations of 2, 4, 6, 8, and 10 mg/ml, respectively. The IC_{50} was found to be 5.94 ± 0.06 µg/ml for "Triphala" and for standard, ascorbic acid it was found to be 2.24 ± 0.02 µg/ml. As shown in Figure 2, DPPH scavenging was increased in a concentration dependent manner compared to ascorbic acid and used as the positive antioxidant control in this investigation.

Reducing power

Iron(III) to Iron(II) reducing activity: The reducing ability of a compound generally depends on the presence of reductants (Duh et al., 1999) which have been exhibiting antioxidative potential by breaking the free radical chain and donating a hydrogen atom (Gordon, 1990). The presence of deductants in "Triphala" extract causes the reduction of the Fe^{3+}/ferricynide complex to the ferrous form. Therefore, the Fe^{2+} can be monitored by measuring the formation of Perl's Purssian blue at 700 nm. Figure 3 shows the reductive capabilities of the "Triphala" extract compared to ascorbic acid. Reducing power of "Triphala" extract ranging from 29.06 ± 0.4 to 7.125 ± 0.7 mg Asc AE/gm.

FRAP

The reducing ability of the extracts was in the range of 407.60 to 695.98 µm Fe (II)/g (Table 2). The antioxidant

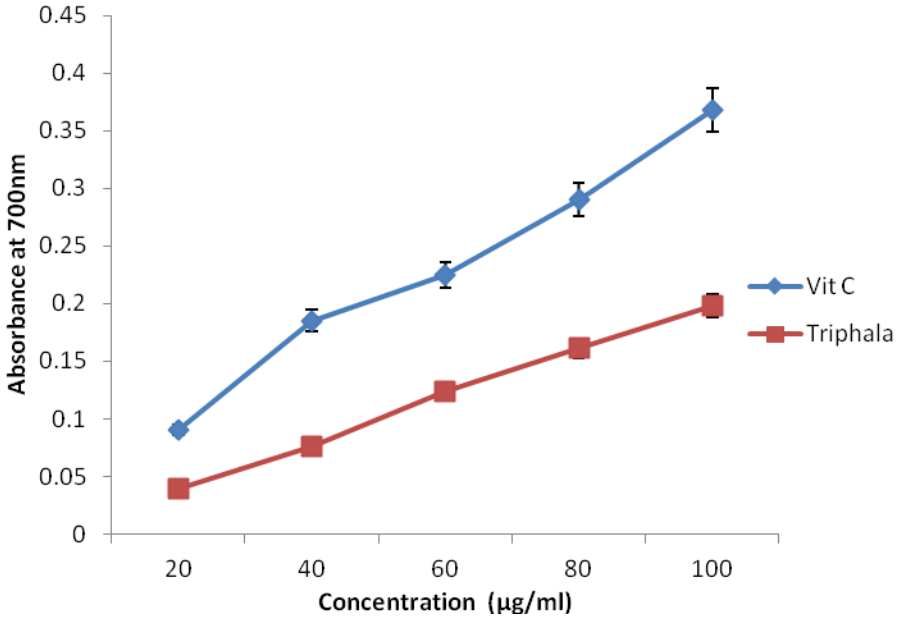

Figure 3. Reducing power activity of Triphala and Vitamin C. Data are presented as Mean± SD.

potentials of the ethanol extracts of "Triphala" was estimated from its ability to reduce TPRZ-Fe(III) complex to TPTZ-Fe(II). The FRAP values for the ethanol extract of "Triphala" was significantly lower than that of ascorbic acid. The ferric reducing/antioxidant power (FRAP assay) is widely used in the evaluation of the antioxidant component in dietary polyphenols (Luximon-Ramma et al., 2005). Antioxidant activity increased proportionally to the polyphenol content. According to recent reports, a highly positive relationship between total phenols and antioxidant activity appears to be the trend in many plant species (Oktay et al., 2003).

Superoxide scavening activity

In the phenazine methosulphate/Nicotinamide adenine dinucleotide-nitroblue tetrazolium (PMS/NADH-NBT) system, superoxide anion is generated using a non-enzymatic reaction of phenazine methosulphate in the presence of NADH and molecular oxygen (Robak and Gryglewski, 1998; Yen and Hsieh, 1998). Superoxide anion reduces NBT into formazan at pH 7.8 at room temperature and formazan generation is followed by spectrophotometry at 560 nm. The decrease of absorbance at 560 nm with antioxidants thus indicates the consumption of superoxide anion in the reaction mixture. In this assay, "Triphala" extract exhibited very strong superoxide anion scavenging activity and the result is presented in Figure 4. The superoxide anion radical-scavenging activity of the extract may be due to the presence of phenolic compounds. The IC_{50} was found to be 42.95 ± 2.07 µg/ml for "Triphala" and for standard ascorbic acid, it was found to be 34.56 ± 2.11 µg/ml. The increase in activity is due to increase in number of phenolic hydroxyl groups in the molecule.

H_2O_2 scavenging activity

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. It can cross cell membranes rapidly and inside the cell. H_2O_2 probably reacts with Fe^{2+} and possibly Cu^{2+} ions to form hydroxyl radical which may be the origin of many of its toxic effects (Miller et al., 1993). It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate. The scavenging activity of the "Triphala" extract is shown in Figure 4. Ascorbic acid was used as the positive control. The IC_{50} was found to be 16.63 ± 2.01 µg/ml for "Triphala" and for standard ascorbic acid, it was found to be 20.77 ± 1.64 µg/ml. "Triphala" scavenged H_2O_2 and this may be attributed to the presence of phenols and tannins which could donate electrons, thereby neutralizing it into water. It was observed that ethanol extracts of "Triphala" inhibition ranged from 59.924 to 49.85% (Figure 5).

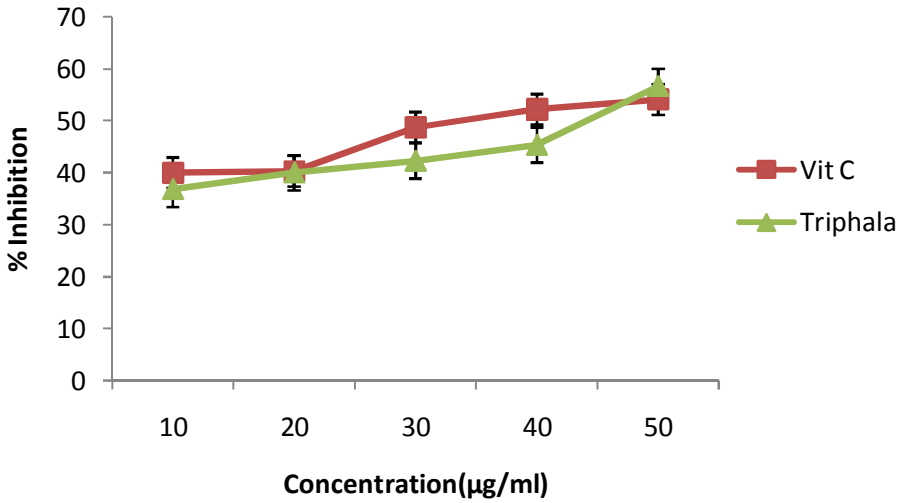

Figure 5. Superoxide Scavenging Assay.

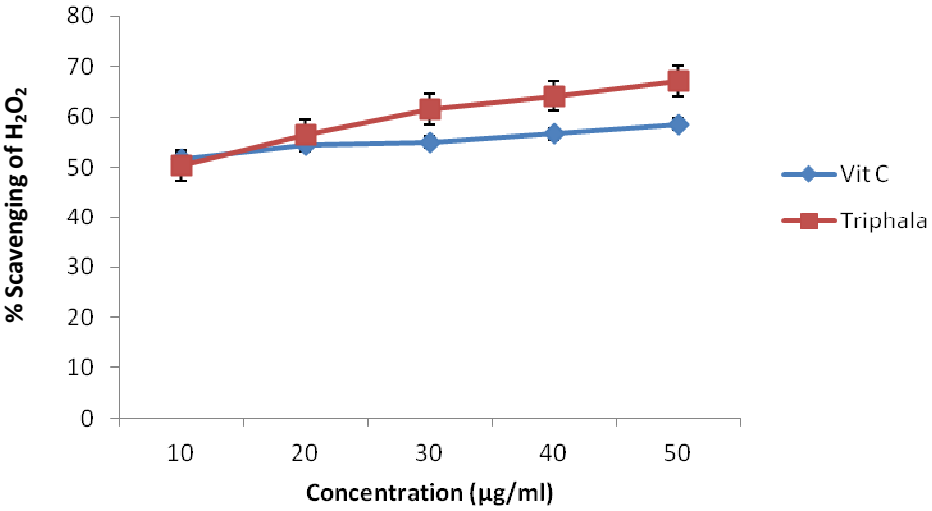

Figure 5. Hydrogen peroxide (H_2O_2) scavenging activity of Triphala and Vitamin C. Data are presented as the percentage of H_2O_2 radical scavenging, Mean± SD.

Nitric oxide radical scavenging assay

Incubation of solutions of sodium nitroprusside in phosphate buffer saline at 25℃ for 150 min resulted in the generation of NO. "Triphala" effectively reduced the generation of NO. The IC_{50} was found to be 32.59 ± 1.54 µg/ml for "Triphala" and 190.46 ± 3.87 µg/ml for ascorbic acid (Table 1). NO radical plays multiple roles in a variety of biological processes which serves as an effector molecule, neuronal messenger, vasodilator, antimicrobial agent, etc (Hagerman et al., 1998). It has been reported to react with $\cdot O_2$ radical to form peroxynitrite radicals (ONOO-) that cause toxicity to bio molecules such as proteins, lipids and nucleic acids (Yermilov et al., 1995). During the process of inflammation, cells of the immune system generate superoxide radicals in which NADPH oxidase plays an important role in induction of vascular complications (Droge, 2002). $\cdot O_2$ further decomposes into singlet oxygen and HO· that result in massive mitochondrial damage. Triphala significantly inhibits generation of NO· and HO· radicals in a dose-dependent manner. These observations further highlight the importance of "Triphala" in preventing physiological deleterious caused by NO· and ·O2 radicals.

Table 1. IC_{50} values of *in vitro* antioxidant activities of Triphala.

Sample	DPPH (µg/ml)	Nitric oxide (µg/ml)	H_2O_2 (µg/ml)	Superoxide Anion (µg/ml)
Triphala	5.94±0.06	32.59±1.54	16.63±2.01	42.95±2.07
Ascorbic acid	2.24±0.02	190.46±3.87	20.77±1.64	34.56±2.11

Data are presented as mean ± SD.

Table 2. Total phenolic content, Total anti oxidant capacity and Ferric reducing activities of Triphala.

Sample	Total phenolic content (mg GAE/g)	Total anti oxidant capacity (mg AscAE/g)	FRAP assay (µM Fe (II)/g)
Triphala	254±8.3	388.9±2.54	1030±18.54
Ascorbic acid	-	-	1615±22.64

Data are presented as mean ± SD.

Conclusion

In the past few years, interest in the search of new natural antioxidants has grown because reactive oxygen species (ROS) production and oxidative stress is linked to many diseases. The use of synthetic antioxidants generally leads to problems of toxicity. Free radical is a molecule with an unpaired electron and is involved in bacterial and parasitic infections, lung damage, inflammation, reperfusion injury, cardiovascular disorders, atherosclerosis, aging and neoplastic diseases (Thomas and Kalyanaraman, 1997). They are also involved in autoimmune disorders like rheumatoid arthritis etc. (Halliwell, 1997; Beckman and Ames, 1998). Therefore, research for the determination of the natural antioxidants source is important.

In this study, we conclude that ethanolic extract of “Triphala” has good antioxidant property and could be attributed to the presence of flavonoids, alkaloids, tannins, saponin glycosides and phenolic compounds. The results of this study show that ethanolic extract of “Triphala” and its constituents can be of use as an easily accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical industry. However, the components responsible for the antioxidative activity of ethanolic extract of “Triphala” are currently unclear. Therefore, it is suggested that further work could be done on the isolation and identification of the antioxidative components in “Triphala” and its constituents.

REFERENCES

Arora S, Kaur K, Kaur S (2003). "Indian medicinal plants as a reservoir of protective phytochemicals". Teratog. Carcinog. Mutagen. Suppl. 1:295-300.

Beckman KB, Ames BN (1998). "The free radical theory of aging matures". Physiol. Rev. 78(2):547-581.

Benzie IF, Strain JJ (1996). "The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay." Anal. Biochem. 239(1):70-76.

Betteridge DJ (2000). "What is oxidative stress?". Metab. 49(2):3-8.

Blios MS (1958). Antioxidant determinantions by the use of a stable free radical. Nature.26:1199-1200.

Droge W (2002). Free radicals in the physiological control of cell function. Physiol. Rev. 82:47-95.

Duh PD, Tu YY, Yen GC (1999). Antioxidant activity of water extract of Harng Jyur (Chrysanthemum moifolium Ramat). Lebensm-Technol. 32:269-277.

Espin JC, Soler-Rivas C, Wichers HJ, Garcia-Viguera C (2000). Anthocyanin-based natural colorants: a new source of antiradical activity for foodstuff. J. Nat. Prod. 48:1588-1592.

Garrat DC (1964). The quantitative analysis of drugs. Chapman and Hall ltd, Japan. Biochem. Anal. Chem. 3:456-458.

Gordon MH (1990). The mechanism of the antioxidant action *in vitro*. In: Food antioxidants, Hudson BJF (Ed.). London, Elsevier. pp. 1-18.

Grice HC (1986). Safety evaluation of butylated hydroxytoluene (BHT) in the liver, lung and gastrointestinal tract. Food Chem. Toxicol. 24:1127-1130.

Hagerman AE, Jones KM, Riedl SGA (1998). High molecular weight plant polyphenolics (tannins) as biological antioxidants. J. Agric. Food Chem. 46:1887-1892.

Halliwell B (1997). Oxygen Radicals and Disease Process. In: Thomas, CE, Kalyanaraman B, (Eds.). The Netherlands Hardwood Academic Publishers. p. 1-14.

Huang D, Ou B, Prior RL (2005). "The chemistry behind antioxidant capacity assays." J. Agric. Food Chem. 53(6):1841-1856.

Jagetia GC, Baliga MS, Malagi KJ, Sethukumar KM (2002). "The evaluation of the radioprotective effect of Triphala (an ayurvedic rejuvenating drug) in the mice exposed to gamma-radiation." Phytomed. 9(2):99-108.

Jagetia GC, Malagi KJ, Baliga MS, Venkatesh P, Veruva RR (2004). "Triphala, an ayurvedic rasayana drug, protects mice against radiation-induced lethality by free-radical scavenging." J. Altern. Complement Med. 10(6):971-978.

Jagetia GC, Rao SK, Baliga MS, Babu KS (2004). "The evaluation of nitric oxide scavenging activity of certain herbal formulations *in vitro*:

a preliminary study." Phytother. Res. 18(7):561-565.
Jayaprakasha GK, Jaganmohan RL, Sakariah KK (2004). "Antioxidant activities of flavidin in different *in vitro* model systems." Bioorg. Med. Chem. 12(19):5141-5146.
Kaur S, Arora S, Kaur K, Kumar S (2002). "The *in vitro* antimutagenic activity of Triphala--an Indian herbal drug." Food Chem. Toxicol. 40(4):527-534.
Kaur S, Michael H, Arora S, Härkönen PL, Kumar S (2005). "The *in vitro* cytotoxic and apoptotic activity of Triphala--an Indian herbal drug." J. Ethnopharmacol. 97(1):15-20.
Kayano S, Kikuzaki H, Fukutsuka N, Mitani T, Nakatani N (2002). Antioxidant activity of prune (*Prunus domestica* L.) constituents and a new synergist. J. Agric. Food Chem. 50:3708-3712.
Kumar P, Kuttan R, Kuttan G (1996). "Radioprotective effects of Rasayanas." Indian J. Exp. Biol. 34(9):848-850.
Lim SN, Cheung PCK, Ooi VEC, Ang PO (2002). Evaluation of antioxidative activity of extracts from a brown seaweed, *Sargassum siliquastrum*. J. Agric. Food Chem. 50:3862-3866.
Liu F, Ooi VE, Chang ST (1997). "Free radical scavenging activities of mushroom polysaccharide extracts." Life Sci. 60(10):763-771.
Lonkar P, Dedon PC (2011). "Reactive species and DNA damage in chronic inflammation: reconciling chemical mechanisms and biological fates." Int. J. Cancer 128(9):1999-2009.
Luo XD, Basile MJ, Kennelly EJ (2002). Polyphenolic antioxidants from the fruit of *Chrysophyllum cainito* L. (Star Apple). J. Agric. Food Chem. 50:1379-1382.
Luximon-Ramma A, Bahorun T, Soobrattee MA, Aruoma OI (2002). "Antioxidant activities of phenolic, proanthocyanidin, and flavonoid components in extracts of Cassia fistula." J. Agric. Food Chem. 50(18):5042-5047.
Maeda S, Omata M (2008). "Inflammation and cancer: role of nuclear factor-kappa B activation." Cancer Sci. 99(5):836-842.
Mehta BK, Shitut S, Wankhade H (1999). *In vitro* antimicrobial efficacy of *triphala*. Fitoterapia. 64:371-372.
Miller MJ, Sadowska-Krowicka H, Chotinaruemol S, Kakkis JL, Clark DA (1993). "Amelioration of chronic ileitis by nitric oxide synthase inhibition." J. Pharmacol. Exp. Ther. 264(1):11-16.
Montecucco F, Pende A, Quercioli A, Mach F (2011). "Inflammation in the pathophysiology of essential hypertension." J. Nephrol. 24(1):23-34.
Nadkarni AK (1976). Indian *Materia Medica*, Popular Press Ltd, Mumbai. 3rd edition. pp. 1308-1315.
Naik GH, Priyadarsini KI, Bhagirathi RG, Mishra B, Mishra KP, Banavalikar MM, Mohan H (2005). "*In vitro* antioxidant studies and free radical reactions of triphala, an ayurvedic formulation and its constituents." Phytother. Res. 19(7):582-586.
Nishimiki M, Appaji N, Yagi K (1972). "The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen." Biochem. Biophys. Res. Commun. 46(2):849-854.
Oktay M, Gulcin I, Kufrevioglu OI (2003). Determination of *in vitro* antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. Lebensm. Wiss. Technol. 36:263-271.
Oyaizu M (1986). Studies on products of browning reaction: Antioxidative activity of product of browning reaction prepared from glucosamine. Japan J. Nutr. 44:307-315.
Prieto P, Pineda M, Aguilar M (1999). "Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E." Anal. Biochem. 269(2):337-341.
Robak J, Gryglewski RJ (1988). "Flavonoids are scavengers of superoxide anions." Biochem. Pharmacol. 37(5):837-841.
Sawa T, Nakao M, Akaike T, Ono K, Maeda H (1999). "Alkylperoxyl radical-scavenging activity of various flavonoids and other phenolic compounds: implications for the anti-tumor-promoter effect of vegetables." J. Agric. Food Chem. 47(2):397-402.
Sell H, Eckel J (2009). "Chemotactic cytokines, obesity and type 2 diabetes: in vivo and *in vitro* evidence for a possible causal correlation?" Proc. Nutr. Soc. 68(4):378-384.
Shahidi F, Wanasundara PK (1992). "Phenolic antioxidants." Crit. Rev. Food Sci. Nutr. 32(1):67-103.
Singh PK (2003). Mycotoxin elaboration in *triphala* and its constituents. Indian Phytopathol. 56:380-383.
Singleton VL, Orthofer R, Lamuela-Raventos RM (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. 299:152-178.
Srikumar R, Jeya PN, Sheela DR (2005). "Immunomodulatory activity of triphala on neutrophil functions." Biol. Pharm. Bull. 28(8):1398-1403.
Tariq M, Hussain SJ, Asif M, Jahan M (1977). "Protective effect of fruit extracts of Emblica officinalis (Gaertn) and Terminalia belerica (Roxb.) in experimental myocardial necrosis in rats." Indian J. Exp. Biol. 15(6):485-486.
Thakur C P, Thakur B, Singh S, Sinha PK, Sinha SK (1988). "The Ayurvedic medicines Haritaki, Amala and Bahira reduce cholesterol-induced atherosclerosis in rabbits." Int. J. Cardiol. 21(2):167-175.
Thomas CE, Kalyanaraman B (1997). In Oxygen Radicals and the Disease Process, Hardwood Academic Publishers, The Netherlands.
Vani T, Rajani M, Sarkar S, Shishoo CJ (1997). Antioxidant properties of the ayurvedic formulation *triphala* and its constituents. Int. J. Pharmacogn. 35:313-317.
Watz B (2008). "Anti-inflammatory effects of plant-based foods and of their constituents." Int. J. Vitam. Nutr. Res. 78(6):293-298.
Wichi HP (1988). Enhanced tumor development by butylated hydroxyanisole (BHA) from the prospective of effect on forestomach and oesophageal squamous epithelium. Food Chem. Toxicol. 26:717-723.
Yen GC, Hsieh CL (1998). Antioxidant activity of extracts from du-zhong (*Eucommia ulmoides*) towards various lipid peroxidation models *in vitro*. J. Agric. Food Chem. 36:3952-3957
Yermilov V, Rubio J, Becchi M, Friesen MD, Pignatelli B, Ohshima H (1995). Formation of 8-nitroguanine by the reaction of guanine with peroxynitrite *in vitro*. Carcinog. 16:2045-2050.