JKIMSU, Vol. 1, No. 2, July-Dec. 2012
ISSN 2231-4261

ORIGINAL ARTICLE

In-vitro Antioxidant Potential of a Herbal Preparation Containing Four Selected Medicinal Plants.

Preeti Padmanabhan[1*], Suresh N. Jangle[1]

[1]*Department of Biochemistry, Rural Medical College, Loni 413736, (Maharashtra) India.*

Abstract:

Background: The therapeutic effects of several plants used in traditional medicine, are usually attributed to their antioxidant properties. *Aim and objective:* To evaluate the *in-vitro* antioxidant potential of herbal preparation a combination of four selected medicinal plants (HP-4) using different experimental models. *Material and Methods:* Polyphenols, flavonoids and flavonols concentrations and antioxidant activity of herbal preparation (HP-4) as compared to butylated hydroxyl toluene (BHT) and á- tocopherol in various experimental models were evaluated. *Results:* The antioxidant activities of HP-4 were concentration dependent in different experimental models and were comparable to activities of BHT and α- tocopherol. *Conclusion:* Polyherbal formulation of HP-4 is better than individual plant extracts.

Keywords: phytochemicals, antioxidants, herbal preparation.

Introduction:

Reactive oxygen species [ROS], sometimes called active oxygen species, are various forms of activated oxygen, which include free radicals such as superoxide ions ($O_2^{-\cdot}$) and hydroxyl radicals ($OH^{\cdot}$), as well as non-free –radical species such as hydrogen peroxide (H_2O_2) [1]. These ROS play an important role in degenerative or pathological processes, such as aging, cancer, coronary heart disease, Alzheimer's disease, neurodegenerative disorders, atherosclerosis, cataracts and inflammation [2].

In living organism various ROSs are formed in different ways, including normal aerobic respiration, stimulated polymorphonuclear leukocytes, macrophages and perioxisomes. These appear to be the main endogenous sources of most of the oxidants produced by cells. Exogenous sources of free radicals include tobacco smoke, ionizing radiation, certain pollutants, organic solvents and pesticides [1]. The term 'antioxidant' refers to the activity of numerous vitamins, minerals and other phytochemicals to protect the damage caused by ROS [3]. Antioxidant defense system scavenges and minimizes free radicals formation. The actions of free radicals are counteracted by antioxidants, either endogenous or exogenous [4]. The therapeutic effects of several medicinal plants are usually attributed to their antioxidant phytochemicals. It has been suggested that there is an inverse relationship between dietary intake of antioxidant rich food and incidence of human disease [1]. Synthetic antioxidant compounds, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are used in processed food but they have side effects. Plant based antioxidants are now preferred to synthetic ones because of safety con-

cerns [5]. Therefore, research regarding antioxidant potential from plant source is important. *Aloe vera* is a gift of nature; it is a miracle being used medicinally for centuries. It belongs to the family Liliaceae, botanically known as *Aloe barbadensis* Mill. *Aloe vera* is commonly known as *Khorpad* in Marathi. It plays an important role due to many medicinal properties in traditional medicine in the treatment of insomnia, burns, ulcerative colitis, psoriasis and wound healing [6]. *Bacopa monniera* Linn family Scrophulariaceae commonly known as *'Brahmi'* is a component of several popular drugs of Ayurvedic system of medicine [7]. In Ayurveda, the plant has been used in the treatment of insanity, epilepsy and hysteria. The other reported activities include sedative, vasoconstrictor and anti-inflammatory [8]. *Zingiber officinale* commonly known as ginger belongs to Zingiberaceae family. Ginger has been used as medicine form Vedic period and is called *"maha aushadhi "* means the great medicine [9]. Some of the uses of ginger are antioxidant, antiemetic, anti-inflammatory, anti-angiogenesis, anti-tumour, and anti-thrombotic [10]. *Moringa oleifera* Lam, Family Moringaceae commonly known as drumstick is cultivated for different purposes such as medicine, vegetable, spice for cooling and cosmetic oil [11]. Various parts of *Moringa* acts as cardiac and circulatory stimulants, possess anti-tumour, anti-inflammatory, anti-ulcer, antispasmodic, hepatoprotective, antibacterial and antifungal activity and are being employed for treatment of different ailments in indigenous system of medicine [12]. In the present study a mixture of 80% alcoholic extract of specific part of plant is evaluated for the *in vitro* antioxidant properties using various experimental models.

Material and Methods:

The leaves of *Aloe vera, Bacopa monniera and Moringa oleifera* and also rhizome of *Zingiber officinale* were collected in from Loni, Maharastra. The herbs were identified by a Professor of Botany, Loni. The leaves and rhizome was shade dried for 4-6 weeks and powdered finely in a mixture and sieved twice to obtain a fine powder.

100 gm of dried powder of leaves of each Aloe *vera, Bacopa monniera, Moringa oleifera* and rhizome of *Zingiber officinale* were separately extracted with Soxhlet extractor using 80 % alcohol till solvent was colourless. The extract was dried till constant weight was obtained. The yield was 25.16% for *Aloe vera,* 16.18% for *Bacopa monniera,* 14.90% for *Moringa oleifera* and 11.68% for *Zingiber officinale.*

25 mg of each extract was mixed together and henceforth labeled as Herbal Preparation (HP-4). This mixture of 25mg each of residue was dissolved in 10ml of methanol, boiled in water bath for 5 minutes, cooled and centrifuged at 4000 rpm for 10 minutes. The clear supernatant was used for evaluating antioxidant properties in various assays.

Phytochemical Analysis:

The herbal preparation was subjected to preliminary phytochemical studies using standard procedures to detect the phytochemicals present.

Estimation of Total Phenolic Compounds:

Total phenolic content was determined by the Folin Ciocalteu method [13].To 0.5ml of 1-5

mg/ml of herbal preparation made up with 0.5ml of distilled water, 0.5 ml of Folin Ciocalteu reagent was added and gently mixed. After 2 minutes 0.5ml of 100mg/ml sodium carbonate was added. The contents were mixed and allowed to stand for 2 hours. The optical density of the blue coloured samples was measured at 765 nm spectrophotometrically. Standard gallic acid of concentration 100-500 microgram/ml was used. The concentration of total phenolics is expressed as milligram of gallic acid) /g of mixture. All determinations were carried out in triplicate.

Estimation of flavonoids:

The method used by Chang *et al* 2002 [14] with slight modifications was followed for estimation of flavonoids. 0.5ml of concentration having 100-500 µg/ml of herbal preparation was mixed with 1ml aluminium trichloride in ethanol (20g/l) and diluted with ethanol to 25 ml. The absorbance at 415 nm was read after 40 minutes at 37°C. Rutin (citrus flavonoids glycoside) a class of flavonoids also called as rutoside; of concentration 0.5mg/ml, 1.0mg/ml, 1.5mg/ml, 2.0mg/ml and 2.5mg/ml was used as a reference compound and absorbance was measured under the same conditions. All determinations were carried in triplicate. The amount of flavonoids in herbal preparation was calculated as milligram of rutin/g of mixture.

Estimations of flavonols:

The content of flavonols was determined by Yermakov *et al* 1987 [15] with slight modifications like reducing the total volume. 0.05 ml of various concentrations (100-500 µg) was treated with 1ml of 2% aluminium trichloride in ethanol and 1ml of 5% sodium acetate. The absorption at 400nm was read after 2.5 hours at 37°C. The same procedure was carried out for 2ml of reference compound rutin for concentration 0.2mg/ml, 0.4mg/ml, 0.6mg/ml, 0.8mg/ml and 1.0mg/ml. All determinations were carried out in triplicate. The content of flavonols was calculated in terms of milligram of rutin /g of mixture.

2, 2' azino-bis (3-ethylbenzothiazoline-6-sulphonate) radical cation decolourization assay [19] :

ABTS radical cation (ABTS +) was produced by reacting 2,2-azinobis-(-3ethyl benzothiozoline-6-sulphonate) ABTS solution (7mM) with 2.45 M ammonium per sulfate and the mixture was allowed to stand in dark at room temperature for 12-16 hours before use. For the study method of Shirwaiker A *et al*, 2006 [16] was used. Different concentrations (200µg -1000 µg) of herbal preparation (0.5ml) were added to 0.3 ml of ABTS solution and the final volume was made up with ethanol to make 1ml. The absorbance was read at 745nm and the experiment was performed in triplicate. Standards BHT and α tocopherol were treated similarly.

Assay of superoxide radical scavenging:

The superoxide radical scavenging activity was based on the capacity of the herbal preparation to inhibit blue formazan formation by scavenging the superoxide radicals generated in riboflavin-light-nitro blue tetrazolium system which was method used by Anandjiwala *et al*, 2008 [17]. The reaction mixture contained 50mM phosphate buffer (pH 7.6), 20µg riboflavin, 12 mM ethylenediamine tetra acetic acid, nitro

blue tetrazolium 0.1mg/3ml, added in that sequence. The reaction was started by illuminating the reaction mixture with different concentrations of herbal preparation (200µg -1000 µg) for 150 seconds. Immediately after illumination, the absorbance was measured at 590 nm and IC_{50} was calculated. Methanol was treated similarly and used for control reading. Standards used were BHT and á- tocopherol [16].
Percent inhibition was calculated by comparing the results of control and test samples using following formula.

$$\% \text{ Inhibition} = \frac{\text{Control absorbance-Test absorbance}}{\text{Control absorbance}} \quad \text{(Equation 1)}$$

Nitric oxide radical scavenging activity:

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacted with oxygen to produce nitrite ions which were measured using Griess reaction method of Singh *et al*, 2008 [18]. 3ml of 10mM sodium nitroprusside in phosphate buffer 0.2M, pH 7.4 was added to 2ml of different concentrations of herbal preparation (200µg - 1000 µg). A similar procedure was repeated with methanol as a blank, which served as control. To 5ml of the incubated sample, 5 ml Griess reagent (1% sulfanilamide, 0.1 % napthylethylenediamine hydrochloride in 2 % H_3PO_4) was added. The absorbance of the chromophore formed was measured at 546nm. All tests were performed in triplicate. Percent inhibition of the nitric oxide generated was measured by comparing the absorbance values of control and test preparation (equation 1). Standards BHT and α- tocopherol were used as reference materials.

Hydroxyl radical scavenging activity:

Hydroxyl radical scavenging activity was measured comparing deoxyribose and herbal preparation for hydroxyl radical generated by the Fe^{3+}-ascorbate – EDTA-H_2O_2 system (Fenton reaction) using the method of Kunchandy and Rao,1990 [19]. The reaction mixture containing 100 µl 2 deoxy -2 ribose (28mM in 20 mM phosphate buffer pH 7.4), 500 µl of herbal preparation and reference compounds BHT & α - tocopherol in phosphate buffer (20mM, pH 7.4), 200 µl of 1.04 mM EDTA & 200 µM ferric chloride ($FeCl_3$) (1:1v/v), 100 µl 1.0mM hydrogen peroxide (H_2O_2) and 100 µl 1.0 mM ascorbic acid was incubated at 37°C for an hour. One ml of 1% thiobarbituric acid and 1.0 ml 2.8% trichloroacetic acid were added and incubated at 100°C for 20 minutes. After cooling absorbance was measured using spectrophotometer at 532 nm against a control preparation containing deoxyribose and buffer. Reaction was carried out in triplicate. Percent inhibition was determined by comparing the results of the test and control samples. Standards BHT and α- tocopherol were used for comparison.

In - Vitro Antioxidant activity using cod –liver oil:

The in-vitro antioxidant activity was determined by method of Lee *et al*, 2002 [20]. Different concentrations of herbal preparation (200µg - 1000 µg) were mixed with diluted 200 µl cod-liver oil source of linoelic acid 25 mg/ml in 99% ethanol and 50 mM phosphate buffer pH 7.4 and incubated at 40°C for a period of 15 minutes. 100 µl of aliquot of above solution was mixed with 3 ml of 70% ethanol, 100 µl of

ammonium thiocyanate (300mg/ml) and 100 µl ferrous chloride (2.45 mg/ml in 3.5% HCL). Incubation was carried out at room temperature for 3 minutes. Absorbance was read at 500 nm. Standards BHT and á-tocopherol were treated similarly. Percent inhibition was calculated using the equation 1.

The Ferric Reducing Ability as a Measure of Antioxidant Power (The FRAP assay) [21]:

The FRAP reagent was prepared by mixing 300mM Acetate Buffer, 10 mM Tripyridyltriazine TPTZ in 40 mM HCL and 20 mM FeCl3. 6H2O in the ratio 10:1:1. Briefly 50µl of different concentrations of herbal preparation (200µg-1000 µg) were added to 1.5ml freshly prepared and pre warmed FRAP reagent at 37°C and incubated at 37°C for 10 minutes. The absorption of blue coloured complex was read against blank using distilled water. BHT and α - tocopherol were used as standards. The absorbance was measured at 593 nm. The data was expressed as mMol ferric ions reduced to ferrous form per litre FRAP. Statistical analysis: The results were expressed as % Inhibition as Mean ±SD of 3 determinations, on applying test for significance $p<0.05$ was considered as statistically significant. IC_{50} values were also calculated for each assay.

Results: The phytochemical analysis of HP-4 showed that it is rich in polyphenols. The polyphenol content was 29.53 ± 0.42 mg/g of gallic acid equivalent. The amount of flavonoids in HP-4 in rutin equivalent was 24.05 ± 0.57 mg/g and the content of flavonols was 17.90 ± 0.24 mg/g.

The ABTS radical scavenging activity was found to be concentration dependent and comparable with BHT and α - tocopherol as shown in Table 1.

The superoxide radical scavenging activity of HP-4 is shown in Table 2. The scavenging effect was comparable to BHT and á-tocopherol and concentration dependent. The IC_{50} values of HP-4 are comparable to BHT and α-tocopherol.

Table 1: ABTS radical scavenging activity of herbal preparation HP-4.

Concentration (µg/ml)	% Inhibition ± SD		
	HP-4	BHT	α -Tocopherol
200	20.11 ± 0.99	17.66 ± 0.104	6.77 ± 0.101
400	22.91 ± 2.08*	25.33 ± 0.113*	17.33 ±0.098*
600	43.23 ± 0.77*	64.67 ± 0.266*	32.11 ±0.093*
800	71.18 ± 0.92*	72.67 ± 0.275*	49.88 ±0.188*
1000	76.92 ± 0.63*	95.00 ± 0.293*	61.44 ±0.285*
IC 50 ((µg/ml)	580	560	610

Value = Mean ±SD of 3 determinations, *significant values and $p< 0.05$

Table 2: Superoxide radical scavenging activity of herbal preparation HP-4.

Concentration (µg/ml)	% Inhibition ± SD		
	HP-4	BHT	α -Tocopherol
200	19.00 ± 0.101	7.26 ± 0.127	18.50 ± 0.730
400	26.00 ± 0.112*	39.53 ± 0.226*	23.67 ± 0.752*
600	42.00 ± 0.204*	59.23 ± 0.149*	33.70 ± 0.707*
800	56.00 ± 0.215*	75.61 ± 0.534*	55.43 ± 0.670*
1000	66.00 ± 0.201*	92.60 ± 0.588*	62.60 ± 0.635*
IC 50 (µg/ml)	500	470	580

Value = Mean ± SD of 3 determinations, *significant values and $p < 0.05$

Table 3. Illustrates the percentage inhibition of nitric oxide generation of HP-4.
HP-4moderately inhibited nitric oxide generation in dose dependent manner.

Table 3: Nitric oxide radical scavenging activity of herbal preparation HP-4.

Concentration (µg/ml)	% Inhibition ± SD		
	HP-4	BHT	α -Tocopherol
200	30.66 ± 0.280	6.93 ± 0.039	22.19 ± 0.571
400	52.38 ± 0.254*	49.46 ± 0.208*	51.46 ± 0.537*
600	57.47 ± 0.236*	61.00 ± 0.278*	61.63 ± 0.507*
800	60.86 ± 0.262*	85.82 ± 0.465*	81.05 ± 0.478*
1000	66.87 ± 0.278*	91.27 ± 0.567*	94.32 ± 0.471*
IC 50 (µg/ml)	420	380	350

Value = Mean ± SD of 3 determinations, * significant values and $p < 0.05$

Table 4. Illustrates the hydroxyl radical scavenging activity of HP-4 which is concentration dependent and comparable to BHT and α- tocopherol.

Table 4: Hydroxyl radical scavenging activity of herbal preparation HP-4.

Concentration (µg/ml)	% Inhibition ± SD		
	HP-4	BHT	α -Tocopherol
200	80.76 ± 0.205	72.06 ± 0.145	56.19 ± 0.141
400	84.65 ± 0.119*	75.84 ± 0.150*	72.06 ± 0.120*
600	85.77 ± 0.220*	77.27 ± 0.250*	73.80 ± 0.100*
800	87.10 ± 0.122*	79.22 ± 0.249*	85.16 ± 0.107*
1000	88.54 ± 0.125*	84.65 ± 0.205*	87.82 ± 0.113*
IC 50 (µg/ml)	350	360	380

Value =Mean ±SD of 3 determinations, * significant values and $p < 0.05$

The in-vitro antioxidant activity of HP-4 using cod-liver oil is shown in Table 5. The antioxidant activity is concentration dependent. HP-4 has better antioxidant activity than BHT as revealed by IC_{50} value.

Table 5: In-vitro Antilipidperoxidation activity of herbal preparation HP-4 using cod liver oil.

Concentration (µg/ml)	% Inhibition ± SD		
	HP-4	BHT	α -Tocopherol
200	26.79 ± 0.723	19.98 ± 0.108	61.69 ± 0.308
400	47.34 ± 0.699*	25.29 ± 0.135*	80.23 ± 0.140*
600	65.32 ± 0.662*	30.51 ± 0.225*	87.69 ± 0.890*
800	87.91 ± 0.621*	65.35 ± 0.221*	93.30 ± 0.764*
1000	91.73 ± 0.587*	89.66 ± 0.331*	99.15 ± 0.680*
IC 50 ((µg/ml)	380	540	220

Value = Mean ±SD of 3 determinations, *significant values and $p < 0.05$

Total antioxidant potential of HP-4 determined by FRAP assay found to be better than BHT. á tocopherol has better antioxidant potential than HP-4. The antioxidant potential was concentration dependent (Table 6).

Table 6: Total oxidation activity of herbal preparation HP-4

Concentration (µg/ml)	mM ferric ions reduced to ferrous ions per litre FRAP reagent		
	HP-4	BHT	α -Tocopherol
200	38.45 ± 0.126	50.92 ± 0.151	23.16 ± 0.363
400	55.75 ± 0.169*	53.10 ± 0.142*	30.46 ± 0.116*
600	56.38 ± 0.146*	80.86 ± 0.199*	37.64 ± 0.103*
800	61.15 ± 0.148*	91.72 ± 0.261*	41.44 ± 0.103*
1000	65.69 ± 0.158*	93.10 ± 0.327*	42.41 ± 0.100*

Value = Mean ±SD of 3 determinations, * significant values and $p < 0.05$

Discussion:

The ABTS assay involves the oxidation of ABTS (2, 2'azinobis -3 ethyl benzothiazoline -6- sulphonate) to form coloured nitrogen centered radical cation $ABTS^+$. It is thus useful for testing coloured aqueous/alcoholic/ hydroalcoholic plant extracts as they do not absorb light at 734 nm [22].

The ABTS chemistry involves direct generation of ABTS radical, monocation with no involvement of any intermediary radical. In this decolourization assay, the radical cation it forms prior to the addition of antioxidant system rather than the generation of the radical taking place in presence of antioxidant. The results obtained imply the activity of the HP-4 by either inhibiting or scavenging the ABTS radicals since both inhibition and scavenging activity of antioxidants towards $ABTS^+$ radicals have

been reported by Rice Evans and Miller 1997 [23].

Thus the activity of HP-4 may be either by inhibiting or scavenging the $ABTS^+$ radicals since both inhibition and scavenging properties of antioxidants towards $ABTS^+$ radicals have been reported by aqueous extract of a polyherbal formulation [24].

Superoxide anion is the first reduction product of oxygen which is measured in terms of inhibition of generation of O_2 [25].

Superoxide dismutase catalyses the dismutation of reactive superoxide anion to oxygen and hydrogen peroxide [26]. The effect of HP-4 in scavenging anions may be due to inhibition of generation of superoxide. Such an effect was reported for ethanolic extract of *B.monnieri* [8, 27, 7].

Nitric oxide (NO) is a free radical produced in mammalian cells and has a regulatory role in various physiological processes. However, excess production of NO may lead to initiation and development of several diseases [28]. Nitric oxide scavenging action which was found to be dose dependent for HP-4 may be due to antioxidant principles in it which may compete with oxygen to react with nitric oxide [29], and thus inhibit the generation of nitrite. Similar mechanism was reported for the antioxidant action of *Sphaeranthus indicus* (Linn) [16].

Hydroxyl radicals are the major active oxygen species causing lipid peroxidation, damage to DNA and proteins [30]. The Fenton reaction generates hydroxyl radicals (OH^-) which degrade the DNA deoxyribose, using Fe^{++} ions as catalyst. Table 4 indicates that HP-4 was capable of reducing oxidative deoxyribose damage in dose dependent manner. Antioxidant activity of ginger extract (*Zingiber officinale*) with respect to hydroxyl radical lipid peroxidation was reported by Stoilova *et al* [31]. These authors are of the opinion that 6-gingerol and its derivatives present in ginger are responsible for such antioxidant activities. Antilipidperoxidation activity was evaluated using cod liver oil as substrate and the effects of HP-4 were found to be dose dependent. Addition of ferrous ions induced the lipid peroxidation in oil as compared to the lipid peroxides formed using oil only. Peroxidation induction by ferrous ions takes place either through ferry-per ferryl complex as suggested by Gutteridge in 1985 [32], or through OH^- radical by Fenton reaction as suggested by Halliwell in 1978 [33].

The results obtained (in this study for HP4) may be attributed to several reasons viz. the inhibition of ferry-per ferryl complex formation, scavenging of OH^- or superoxide radical or by changing the ratio of Fe^{+++} to Fe^{++}, reducing the rate of conversion ferrous or ferric by chelation of the ion itself [34]. According to Diplock 1997 [35], the antioxidant action may be attributed to various mechanisms, some of which may be prevention of chain initiation, decomposition of peroxides, prevention of continued hydrogen abstraction and radical scavenging action.

The reducing capacity of the compound/compounds in a mixture serve as a significant indicator of its potential antioxidant activity [14]. The reducing capacity of HP-4 was concentration dependent and was better than á-tocopherol (Table 6).

Phenolic compounds are powerful chain breaking antioxidants [36]. It is reported that phe-

nolic compounds contribute directly to antioxidant action [37].

HP -4 is rich in polyphenol, flavonol and flavonoids. Antioxidants are specific to the species of reactive oxygen [38]. Some of them enhance the endogenous defense system enzymes involved in metabolizing these moieties. Some directly interact with superoxides, hydroxyl radicals, singlet oxygen, hydrogen peroxides etc [39]. The phytochemicals may chelate the transition metals and inhibit the formation of free radicals at the initial level itself [40].

Hence the various degree of inhibition of various oxidants can be seen in this aspect in the various scavenging models used in this study.

It is reported that the crude extracts of plants are pharmacologically more active than their isolated active principles due to the synergistic effects of various phytochemicals present in the whole extract [41]. Synergistic effects of ayurvedic formulation Triphala was reported for antioxidant activities of individual components of Triphala, Vani *et al* 1997 [42]. It has been reported that administration of *C.sativus*, *A.sativum* and *C.longa* together have shown increase in antigenotoxic effects against cyclophosphamide induced genotoxicity in mice against administration of individual agents separately, Premkumar *et al* 2004 [43].

Synergistic and supraadditive effect of STW 5 (Iberogast®) containing nine different plant extracts on gastrointestinal remedy [44] and on radical scavenging and anti-inflammatory properties of STW 5 (Iberogast®) and its components is observed [45].

Hence we believe that the poly herbal formulation HP-4 is better than individual plant extracts and the same will be further investigated for antihepatotoxic effects using different experimental animal models.

References:

1. Yildrim A, Oktay M, Bilaloglu V. The antioxidant activity of leaves of *Cydonia vulgaris*. *Turk J Med Sci* 2001; 31: 23-27.
2. Huang DH, Chen C, Lin C, Lin Y. Antioxidant and antiproliferative activities of water spinach (*Ipomoea aquatica* Forsk.) constituents. *Bot Bull Acad Sci* 2005:46:99-106.
3. Khilifi S, El-Hachimi Y, Khalil A, Es-Safi N, Belahyan A, Tellal R, El-Abbouyi A. *In -vitro* antioxidant properties of *Salvia verbenaca* L. hydromethanolic extract. *Indian J Pharmacol* 2006:38(4)-276-280.
4. Jain A, Singhai AK, Dixit VK. *In-vitro* evaluation of *Tephrosia purpurea* Pers. for antioxidant activity. *J Nat Rem* 2006; 6/2:162-164.
5. Akinmoladun AC, Ibukun EO, Afor E, Akinrinlola BL, Onibon TR, Akinboboye AO, Obutor EM. Farombi EM. Chemical constituents and antioxidant activity of *Alstonia boonei*. *Afr J Biotechnol* 2007; 6(10):1197 – 1201.
6. Matti MG, Al-Almeen SA, Rashed SH. Some biochemical effects of *Aloe vera* leaves on tissues in normal mice. *Iraqi J Vet Sci* 2010; 24 (2):93-97.
7. Tripathi YB, Chaurasia S, Tripathi E, Upadhyay A, Dubey GP. *Bacopa monnieri* Linn as an antioxidant: Mechanism of action. *Ind J Exp Biol* 1996;

34:523-526.

8. Ghosh T, Maity TK, Das M, Bose A, Dash DK. *In-vitro* antioxidant and Hepatoprotective activity of Ethanolic extract of *Bacopa monnieri*. Linn. aerial parts. *Iran J Pharmacol Ther* 2007; 6(1):77-85.
9. Kalpagam P, Nirmala K. Ginger: its role in xenobiotic metabolism. *I C M R Bulletin* 2003; 33 (6); 57-63.
10. Minaiyen M, Ghannadi A. Karim zadeh A, Anti-ulcerogenic effect of Ginger (rhizome of *Zingiber officinale* Roscoe) on cysteimine induced duodenal ulcer in rats. *DARU* 2006; 14 (2):97-101.
11. Goyal BR, Agrawal BB, Goyal RK, Mehta AA. Phytopharmacology of *Moringa oleifera* Lam. an overview. Natural Product Radiance 2007: 6(4):347-353.
12. Nepolean P, Anitha J, Emilin Renitta R. Isolation, analysis and identification of phytochemical and antimicrobial activity of *Moringa oleifera* Lam. *Current Biotica* 2009; 3 (1):33-39.
13. Folin O, Ciocalteau V. On tyrosine and tryptophan determinations in proteins. *J Biol Chem* 1927; 27:627-650.
14. Chang C, Yang M, Wen H. Estimation of total flavonoids content in propolis by two complementary colorimetric methods. *J Food Drug Anala* 2002; 10:178-182.
15. Ermakov AI, Arasimovich VV, Smirnova-Ikonnikova MI, Yarosh NP, Lukovnikova GA. Methods of Biochemical analysis of Plants. 1972: 456.
16. Shirwaiker A, Prabhu KS, Punitha ISR. *In –vitro* antioxidant studies of *Sphaeranthus indicus* (Linn). *Ind J Exp Biol* 2006; 44: 993-996.
17. Anandjiwala S, Bagul MS, Parabia M, Rajani M. Evaluation of free radical scavenging activity of an ayurvedic formulation *Panchvalkala*. *Indian J Pharm Sci* 2008: 70(1):31-35.
18. Singh R, Singh N, Saini BS, Rao HS. *In –vitro* Antioxidant activity of pet. ether extract of black pepper. *Indian J .Pharmacol* 2008; 44:147-151.
19. Kunchandy E, Rao MNA. Oxygen radical scavenging activity of curcumin. *Int J Pharm* 1990; 58: 237 - 240.
20. Lee JC, Kim KR, Jim KJ. Antioxidant property of an extract of the stem of *Opuntia ficus* Var. *sabotes*. *J Agric Food Chem* 2002; 50:6490-6496.
21. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power'. The FRAP assay. *Anal Biochem* 1996; 239:70-76.
22. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Evans C. Antioxidant activity applying an improved ABTS radical cation decolourization assay. *Free Rad Biol Med* 1999; 26: 1231-1237.
23. Rice Evans CA, Samson J, Bramley PM, Hallowa DE. Why do we expect carotenoids to be antioxidants *in vivo*?*Free Rad Res* 1997; 26: 381- 390.
24. Shirwaiker A, Aswatha Ram HN, Mohpatra P. Antioxidant and antiulcer activity of aqueous extract of polyherbal formulations. *Ind J Expt Biol* 2006; 44:474-480.

25. Kamalakkannan N, Stanley MPP. Effect of *Aegle marmelos* fruit extract on tissue antioxidants in streptozotocin diabetic rats. *Ind J Exp Biol* 2003; 41: 1285-1288.
26. Blois MS. Antioxidant determinations by the use of stable free radical. Nature1958; 26:1199-1200.
27. Meena H , Pandey HK, Pandey P, Arya MC, Ahmed Z. Evaluation of antioxidant activity of two important memory enhancing medicinal plants *B.monnieri* and *C. asiatica. Indian J Pharmacol* 2012; 44:114-117.
28. Gibanananda R, Husain SA. Oxidants, antioxidants and carcinogenesis. *Ind J Exp. Biol* 2002; 40:1213-1232.
29. Ilalenti A, Moncada S, Di Rosa M. Modulation of adjuvant arthritis by endogenous nitric oxide. *Brit J Pharmacol* 1993; 110: 701-706.
30. Spencer JPE, Jenner A, Auroma OI. Intense oxidative DNA damage promoted by γ - DOPA and its metabolites, implications for neurogenerative disease. *FEBS Lett* 1994; 353: 246-250.
31. Stoilova I, Krastanov A, Stoyanova A, Dener P, Gargova S. Antioxidant activity of ginger extract (*Zingiber officinale) Food Chem* 2007; 102:764-770.
32. Gutteridge JMC. Age pigments and free radicals: Fluorescent lipid complexes formed by iron and copper containing proteins. *Biochem Biophysics Acta,*1985; 834: 144-148.
33. Halliwell B. Superoxide dependent formation of hydroxyl radicals in the presence of iron chelates. *FEBS Lett* 1978; 92: 321-326.
34. Braughler JM, Ducan CA, Chase LR. The involvement of iron in lipid peroxidation: Importance of ferrous to ferric ratio inhibition. *J Biol Chem* 1986; 261: 10282- 10289.
35. Diplock AT. Will the 'Good Fairies' please prove to us that Vit E lessens human degeneration disease?. *Free Rad Res* 1977; 27: 511- 532.
36. Shahidi F, Wanasundara PD. Phenolic antioxidants. *Cri Rev Food Sci Nutr* 1992; 32 67-103.
37. Duh PD, Tu YY, Yen GC. Antioxidant activity of water extract of *Chrysanthemum morifolium. Leben Smittel –Wisschaft and Technologic* 1999; 32: 269-277.
38. Slatter TF. Oxygen free radical and tissue damage 1979. Ciba Foundation Symposium 65, (Experta Medica Amsterdam, Oxford) New York 143-162.
39. Aruoma OI, Halliwell B, Gajewshi E, Dizdaroglu M. Damage to the bases in DNA induced by hydrogen peroxide and ferric iron chelates. *J Biol Chem* 1989; 264: 20509-20512.
40. Gordon MN. The mechanism of antioxidants action *in –vitro* 1990. In Hudson BJF Ed. Food antioxidants, London, Elsevier Applied Science: 1-18.
41. Hamberger M, Hastettman K. Bioactivity in plants: The link between phytochemistry and medicine. *Phytochemistry* 1991; 30: 3864-3874.

42. Vani T, Rajani M, Sarkar S, Shishoo CJ. Antioxidant properties of the ayurvedic formulation of Triphala and its constituents. *Int J Pharmacognosy* 1997; 35: 313-317.
43. Premkumar K, Kavitha S, Sathiya TS, Ramesh A. Interactive effects of saffron with garlic and curcumin against cyclophosphamide induced genotoxicity in mice. *Asia Pac J Clin Nutr* 2004; 13:292-294.
44. German I, Hagelauer D, Kelber O,Vinson B, Laufer S,Weiser D, Heinle H. Antioxidative properties of the gastrointestinal phyto pharmaceutical remedy STW-5 (Iberogast^{R}). *Phytomedicine* 2006; 13 su: 45-48.
45. Schempp M ,Weiser D, Kelber O, Elstner EF. Radical scavenging and anti-Inflammatory properties of STW-5 (Iberogast®) and its components. *Phytomedicine* 2006; 13 su : 36-44

**** Author for Correspondence:*** *Miss. Preeti Padmanabhan, Department of Biochemistry, Rural Medical College, Loni- 413736, District –Ahmednagar,(Maharashtra) India. Cell no: 9860494394, E-mail: preetipadmanabhan@ gmail.com*,