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Antioxidant and Antilipid Peroxidation Potential of Polyherbal Formulation

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Abstract

The polyherbal formulation was designed with *Tinospora cordifolia, Adathoda vasaka, Stevia rebaudiana, Pterocarpus marsupium, Withania somnifera, Tridax procumbens, Boerhaavia diffusa* and *Syzygium cumini*. The formulation was subjected to bioconversion for 40 days with yeast. This bio-converted PHF contains 10.80 mg ml^{-1} of reducing sugars, 8.84 mg ml^{-1} of total phenol including 2.36 mg ml^{-1} of total flavonoids, 1.02 mg ml^{-1} of gallic acid and 4.73 mg ml^{-1} of tannic acid as well as 0.15 mg ml^{-1} of ascorbic acid. The formulation exhibited lower IC_{50} values for DPPH radical scavenging (99.09 µg ml^{-1}), H_2O_2 radical scavenging (99.09 µg ml^{-1}) and Iron chelation (99.09 µg ml^{-1}) which indicate in good antioxidant potential of PHF, while higher IC 50 values were recorded with nitric oxide radical scavenging (575.96 µg ml^{-1}) and hydroxyl radical scavenging (350.79 µg ml^{-1}) assay models. The PHF also showed good anti-lipid peroxidation potential in goat liver (IC_{50} value 73.87 µg ml^{-1}) and human erythrocyte (IC_{50} value 72.40 µg ml^{-1}) models.

Keywords: polyherbal formulation, antioxidant, lipid peroxidation, goat liver, human erythrocytes.

INTRODUCTION

Technological innovations and advancements made human life easier, but at the same time these advanced facilities have increased competition for incentives based on performance, which lead to stress conditions. It is well documented that the excess stress leads to embarrassment as well as mental ability for consistent thoughts. It also lowers the immunity, which can show the way to pathological conditions [1]. One of the reasons behind modern diseases is oxidative stress, which originates due to imbalance between formation and neutralization of pro-oxidants [2, 3]. Diseases caused due to life style and stress conditions include heart diseases, cancer, rheumatoid arthritis and diabetes.

To defend the body from such ill effects, human body has evolved a highly developed and multifaceted antioxidant defensive mechanism consisting of primary and secondary defensive mechanisms. The antioxidant enzymes are acting as primary defense and catalytically remove the free radicals produced due to oxidative stress from body to maintain healthy life [4]. On the other hand, the secondary defensive mechanism comprises of antioxidants, which are scavenger of free radicals. Such antioxidants are available vegetables and fruits, which include polyphenols, flavonoids and vitamins. These compounds work as antioxidants and scavenge free radicals; repairs the damages caused due to stress and rejuvenate normal physiological processes in humans.

MATERIALS AND METHODS

The polyherbal medicine was formulated with 8 herbs viz. *Tinospora cordifolia, Adathoda vasaka, Stevia rebaudiana, Pterocarpus marsupium, Withania somnifera, Tridax procumbens, Boerhaavia diffusa* and *Syzygium cumini*. The formulation was subjected to bioconversion for 40 days with yeast.

Estimation of reducing sugar: Reducing sugars were estimated with DNSA reagent [5]. The sample (0.2, 0.4, 0.6, 0.8 and 1ml) was mixed with1.0ml of Dinitrosalycylic acid (DNSA) reagent. Reactions were incubated exactly for 10 min in boiling water bath and after incubation the final volume in each tube was made to 10 ml with distilled water. The reaction mixture in control tube was prepared without any and absorbance was measured at 540nm on UV-vis spectrophotometer (Shimadzu). Glucose at concentrations of 100 to 500 µg was to prepare standard curve.

Estimation of Total Phenols: The content of total phenolics of polyherbal formulation (PHF) was estimated with Folin-Ciocalteu reagent [6] with slight modifications. Diluted 0.2, 0.4, 0.6, 0.8, 1ml of PHF sample was mixed with 0.5 ml of Folin-Ciocalteau reagent. After 3 minutes of incubation at room temperature 2 ml of 1.89 M Na_2CO_3 solution was added to each tube, mixed thoroughly and incubated in boiling water bath (1 minute). The blue colour developed in reaction mixture was read at 650 nm on UV-Vis spectrophotometer (Shimadzu-1700). Catechol was used to prepare the standard curve.

Estimation of total flavonoids: The aluminum chloride method was used for flavonoids estimation [7]. The PHF sample 0.2, 0.4, 0.6, 0.8 and 1ml was separately mixed with 1.5 ml of methanol, 0.1 ml of 0.75 M Aluminum Chloride, 0.1 ml of 1 M potassium acetate, and 3 ml of distilled water. The reaction mixture was kept at room temperature for 30 min. The absorbance of the reaction mixture was measured at 450 nm on UV-visible spectrophotometer (Shimadzu-1700).The calibration curve was prepared with quercetin.

Estimation of Ascorbic acid: Ascorbic acid was estimated by titrimetric method with 2, 6 dichlorophenol indophenols [8]. Exactly 5 ml of sample was mixed with 10 ml of 4% oxalic acid and titrated against 2, 6 dichlorophenol indophenol (0.01 M) prepared in 0.02M sodium bicarbonate solution. Ascorbic acid 0.5 mg ml^{-1} was used as standard and values are expressed as mg ml^{-1} of actual sample**.**

Estimation of Gallic and Tannic acid: Gallic and tannic acid content of PHF was estimated by spectrophotometric method [9]. Dilute sample was prepared by dissolving 1 ml PHF in 100 ml methanol. Sample was vigorously mixed on vertex mixture and read at 254.6 nm and 293.8 nm for Gallic and tannic acid against reagent blank consisting 2 ml methanol on UV-visible spectrophotometer (Shimadzu-1700). Concentration of tannic and gallic acid was estimated with the equations given below and values are expressed as mg ml-1 of actual sample.

Conc. of tannic acid (µg mL−1) = 34.41 (A293.8) – 6.98 (A254.6)
Conc. of gallic acid (µg mL−1) = 21.77 (A254.6) – 17.17 (A293.8)

Free Radical Scavenging Activity: Free radical scavenging activity was determined by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) [10] with slight modifications. The 3 ml of assay mixture contained appropriately diluted (25 to 125 µg ml^{-1}) PHF samples in 2.9 ml of methanol and 0.1 ml of 1 mM DPPH. The reactions were started by adding 0.1 ml of 1mM DPPH solution. The reaction mixtures were left to stand for 30 min and absorbance was measured at 517 nm. Blank was carried out without PHF and ascorbic acid was used as a standard. The percentage inhibition was determined by following equation:

% Inhibition= $[(A_0 – A_1) / A_0 \times 100]$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the test sample.

The IC_{50} value (concentration of a sample, which is required to scavenge exact 50% of free radicals) was also calculated from the data obtained.

Nitric oxide radical scavenging: Sodium nitroprusside 5 mM was prepared in phosphate buffer (0.1 M; pH 7.4). Various concentrations (25 to 125 µg ml^{-1}) of PHF were prepared in 1 ml of distilled water in different test tubes and to that 0.3 ml of 5mM sodium nitroprusside was added. The test tubes were incubated at 25 ˚C for 3 hours and then 0.5 ml of Griess reagent was added to each tube. The absorbance was read at 546 nm. Blank was carried out without PHF and ascorbic acid was used as a standard [11].

The percentage inhibition was determined by following equation:

% Inhibition= $[(A_0 – A_1) / A_0 \times 100]$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the test sample.

The IC_{50} value (concentration of a sample, which is required to scavenge exact 50% of Nitric oxide radicals) was also calculated from the data obtained.

Hydroxyl radical scavenging activity: Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and test compounds for hydroxyl radicals generated from Fe3+, ascorbate, EDTA and H_2O_2 system (Fenton reaction). Hydroxyl radical attacks deoxyribose, which eventually results in formation of thiobarbituric acid reacting substance (TBARS) [12]. The total 3 ml of reaction mixture contained 2-deoxy-2-ribose (2.8mM), KH_2PO_4-KOH buffer (20mM, pH 7.4), $FeCl_3$ (100µM), EDTA (100µM), H_2O_2 (1.0mM), ascorbic acid (100µM) and different concentrations of PHF (25 to 125 µg ml^{-1}). After incubation for 1 hour at 37^0 C exactly 1 ml of 171.37 mM trichloroacetic acid and 1 ml of 69.37 mM aqueous thiobarbituric was added and mixture was incubated at 100^0 C for 20 min to develop pink colour. After cooling, absorbance was measured at 532nm against a control sample containing deoxyribose and buffer. Blank was carried out without PHF and ascorbic acid was used as a standard. Percentage inhibition was calculated and concentration needed for 50% (IC_{50}) inhibition was determined.

Hydrogen Peroxide Radical Scavenging Activity: The PHF to scavenge H_2O_2 was determined as per the method suggested by Jayaprakasha et al. [13]. Hydrogen peroxide solution (20 mM) was prepared in phosphate buffered saline (PBS) (pH 7.4). The total 3 ml of reaction mixture contained various concentrations (25 to 125 µg ml^{-1}) of PHF and 2.0 ml of H_2O_2 solution in PBS. Absorbance of H_2O_2 was measured at 230nm. Blank was carried out without PHF and ascorbic acid was used as a standard. Percentage inhibition was calculated and concentration needed for 50% (IC_{50}) inhibition was determined.

Reducing Power Assay: The Fe_3^+ reducing power of PHF samples were determined by the method of Oyaizu [14] with a slight modification. The 3 ml reaction mixture contained phosphate buffer (100 mM; pH 6.6) various concentrations (25 to 125 µg ml^{-1}) of PHF and 3.04 mM Potassium ferricyanide. The reaction tubes were incubated in water bath at 50°C for 20 min. The reactions were stopped by adding 0.61 M TCA and centrifuged at 800 x g for 10 min. The 1.5 ml of supernatant was mixed with 1.5 ml distilled water to that 0.1 ml of 6.17 mM $FeCl_3$ was added and incubated for 10 min. Absorbance was measured at 700 nm against an appropriate blank solution. Blank was carried out without PHF and ascorbic acid was used as a standard. All tests were performed twice. A higher absorbance of the reaction mixture indicated greater reducing power.

Iron chelating activity assay: The method of Benzie and Strain [15] was adopted for the assay. The principle is based on the formation of O-Phenanthroline-Fe2+ complex and its disruption in the presence of chelating agents. The reaction mixture containing 1 ml of 2.52 mM O-Phenanthroline in methanol, 1 ml of 200µM ferric chloride and various concentrations of PHF (ranging from 25 to 125 µg ml^{-1}) in a final volume of 3 ml was incubated for 10 minutes at ambient temperature. The absorbance of these reaction mixtures was measured at 510 nm. Blank was carried out without PHF and EDTA was used as a classical metal chelator. The experiment was run in triplicates.

Antilipid peroxidation assay with goat liver: Lipid peroxidation of goat liver tissues was measured in reaction mixture with thiobarbituric acid (TBA) in acid medium [16]. Healthy goat liver was collected from mutton (butcher) shop in an ice bag and taken to the laboratory. Liver was washed with the physiological saline for 3 times. Exactly 5 g liver tissue was weighed and homogenised it in saline buffer with tissue homogenizer. The preparation was filtered and the filtrate was used for lipid peroxidation assay. Each reaction mixture in final volume of 3 ml contained, phosphate buffered saline (10 mM Sodium Phosphate buffer pH 7.4 supplemented with 0.15 M NaCl), 0.5 ml of tissue homogenate, 10 mM of H_2O_2 and different concentrations of PHF (ranging from 25 to 125 µg ml^{-1}). Similarly a control was prepared with H_2O_2 and liver tissues but without PHF, and adjusted to a final volume with phosphate buffered saline. Simultaneously respective PHF samples were also prepared in buffer without H_2O_2 and liver tissues to eliminate interference of sugars. These assay mixture were incubated for 30 minutes at 37 ^oC in water bath. After incubation, 3 ml 2.83 M TCA was added to each assay mixture for de-proteinization. The assay mixtures were then centrifuged at 5000 RPM for 10 min. Supernatant (2 ml) was taken in fresh test tube and was mixed with 0.5 ml of 62.43 mM TBA and heated at 100 ^oC for 10 min. The absorbance of the coloured product was measured at 532 nm. The percentage inhibition of thiobarbituric acid reacting substance (TBRS) was determined by following equation:

% Inhibition= $[(A_c - A_t) / A_c \times 100]$

Where A_c is the absorbance of the control at 532 nm and A_t is the absorbance of the test sample at 532 nm.

The IC_{50} value (concentration of a sample, which is required to inhibit TBRS by 50% was also calculated from the percent inhibition values.

Antilipid peroxidation assay with human erythrocytes: Lipid peroxidation of erythrocyte membranes was measured in reaction mixture with thiobarbituric acid (TBA) in acid medium [16]. Human blood was obtained from healthy volunteers and was sampled into natrium citrate. The cells were separated by centrifugation at 2500 g for 10 minutes. The erythrocytes were washed two times with same volume of physiological saline. Each reaction mixture in final volume of 3 ml contained, phosphate buffered saline (10 mM Sodium Phosphate buffer pH 7.4 supplemented with 0.15 M NaCl), 0.2 ml of erythrocytes, 10 mM of H_2O_2 and different concentrations of PHF (ranging from 25 to 125 µg ml^{-1}). Similarly a control was prepared with H_2O_2 and erythrocytes but without PHF, and adjusted to a final volume with phosphate buffered saline. Simultaneously respective PHF samples were also prepared in buffer without H_2O_2 and liver tissues to eliminate interference of sugars. These assay mixture were incubated for 30 minutes at 37 ^oC in water bath. After incubation, 3 ml 2.83 M TCA was added to each assay mixture for de-proteinization. The assay mixtures were then centrifuged at 5000 RPM for 10 min. Supernatant (2 ml) was taken in fresh test tube and was mixed with 0.5 ml of 62.43 mM TBA and heated at 100 ^oC for 10 min. The absorbance of the coloured product was measured at 532 nm. The percentage inhibition of thiobarbituric acid reacting substance (TBRS) and IC_{50} value were determined as mentioned in goat liver assay.

RESULT AND DISCUSSION

The estimated values for phytoconstituents of PHF are given table -1. Data show that PHF contains 10.80 mg ml^{-1} of reducing sugars, 8.84 mg ml^{-1} of total phenol including 2.36 mg ml^{-1} of total flavonoids, 1.02 mg ml^{-1} of gallic acid and 4.73 mg ml^{-1} of tannic acid as well as 0.15 mg ml^{-1} of ascorbic acid.

Results on percentage of DPPH and Nitric oxide radical scavenging activities of PHF are presented in table 2. The PHF exhibited a maximum DPPH scavenging activity of 63.04% at 125 µg ml^{-1} whereas for ascorbic acid (standard) was found to be 84.45% at 125 µg ml^{-1}. The IC_{50} of the PHF and ascorbic acid were found to be 99.09 µg $ml^{-1}$and 49.74 µg ml^{-1} respectively. Data on nitric oxide radical scavenging activity clearly indicate that PHF has significantly less (11.11 %) nitric oxide radical scavenging activity as compared to ascorbic acid (82.52 %) at 125 µg ml^{-1} and hence great difference in IC 50 values.

Table-1: Amount of phyto-constituents in polyherbal formulation

Phyto-constituents	Reducing sugar	Total Phenols	Total flavonoids	Tannic acid	Gallic acid	Ascorbic acid
Amount (mg ml^{-1})	10.80	8.84	2.36	4.73	1.02	0.15

Table-2: Effect of PHF on *In-vitro* DPPH and nitric oxide radical scavenging activity

Polyherbal formulation/ Standard (µg ml^{-1})	DDPH Scavenging Activity (% of radical scavenging)		Nitric oxide radical scavenging (% of radical scavenging)	
	Polyherbal formulation	Ascorbic acid	Polyherbal formulation	Ascorbic acid
25	11.59	44.67	1.90	40.79
50	25.36	67.89	4.61	47.37
75	39.86	77.53	6.64	56.50
100	52.17	81.28	9.08	71.50
125	63.04	84.45	11.11	82.52
IC_{50} value (µg ml^{-1})	99.09	49.74	575.96	54.02

Table-3: Effect of PHF on *In-vitro* hydroxyl and hydrogen peroxide radical scavenging activity

Polyherbal formulation / Standard (µg ml^{-1})	Hydroxyl radical scavenging activity (% of radical scavenging)		Hydrogen Peroxide Radical Scavenging Activity (% of radical scavenging)	
	Polyherbal formulation	Ascorbic acid	Polyherbal formulation	Ascorbic acid
25	3.76	32.11	28.62	43.80
50	7.44	60.72	52.08	65.39
75	10.29	70.67	72.23	78.49
100	14.05	79.83	87.52	85.02
125	17.13	87.58	97.14	91.31
IC_{50} value (µg ml^{-1})	350.79	53.42	53.01	48.36

Table-4: Effect of PHF on *in-vitro* iron chelation and reducing power assay.

Polyherbal formulation / Standard (µg ml^{-1})	Iron chelating assay (% Iron Chelation)		Reducing Power Assay (Increase in Abs at 700 nm)	
	Polyherbal formulation	EDTA	Polyherbal formulation	Ascorbic acid
25	12.77	14.78	0.005	0.499
50	27.66	30.34	0.025	0.779
75	39.36	44.12	0.060	0.968
100	48.94	58.72	0.102	1.039
125	55.32	70.18	0.135	1.074
IC_{50} value (µg ml^{-1})	99.75	85.51	-	-

Table -5: Anti-lipid peroxidation effect of PHF on in human erythrocytes and goat liver tissues.

Polyherbal formulation / Standard (µg ml^{-1})	Goat liver assay (% inhibition)		Erythrocytes Assay (% inhibition)	
	Polyherbal formulation	Ascorbic acid	Polyherbal formulation	Ascorbic acid
00	0.00	0.00	0.00	38.98
25	28.70	39.49	18.18	50.48
50	41.54	50.45	38.52	62.18
75	50.91	62.42	50.96	75.02
100	56.50	74.92	69.38	85.92
125	60.42	86.52	75.60	38.98
IC_{50} value(µg ml^{-1})	73.87	32.63	72.40	34.27

Data on percentage of hydroxyl and hydrogen peroxide radical scavenging activities of PHF are given in table 3. The PHF revealed a maximum hydroxyl radical scavenging activity of 17.13 % at 125 µg ml^{-1} whereas for ascorbic acid (standard) was found to be 87.58% at 125 µg ml^{-1}. The IC_{50} of the PHF and ascorbic acid were found to be 350.79 µg ml^{-1}and 53.42 µg ml^{-1} respectively. Data on H_2O_2 radical scavenging activity clearly indicates that PHF has significantly more i.e. 97.14 % H_2O_2 radical scavenging activity as against 82.52 % at 125 µg ml^{-1} of ascorbic acid with IC 50 values of 53.01 µg ml^{-1} and 48.46 µg ml^{-1} and respectively. Hydrogen peroxide (H_2O_2) itself is not very particularly reactive with most biologically important molecules, but is acts as an intracellular precursor of hydroxyl radicals, that are very toxic to the cell [17]. The decreased absorbance of reaction indicates increased reduction of H_2O_2 and hydroxyl radical production. PHF scavenged hydrogen peroxide which can be attributed to the presence of phenolic groups that may perhaps donate electrons to hydrogen peroxide, thereby neutralizing it into water [18].

Result values on percentage of iron chelation by PHF are given in table 4. The PHF showed 55.32 % iron chelation at 125 µg ml^{-1} whereas for ascorbic acid (standard) exhibited 70.18 % chelation at 125 µg ml^{-1}. The IC_{50} of the PHF and ascorbic acid were found to be 99.75 µg ml^{-1}and 85.51 µg ml^{-1} respectively. Iron is essentially is required for oxygen transport, respiration and activity of many enzymes. But, it is an extremely reactive metal which catalyzes oxidative changes in lipids, proteins and other cellular components [19]. Iron is responsible for lipid peroxidation through the Fenton and Haber-weiss Reaction [20] and decomposes the lipid hydroxide into peroxyl and Alkoxyl radicals; these are actually responsible for continuation of the chain reactions. Reducing power assay method is based on the principle that substances, which have reduction potential, react with potassium ferricyanide (Fe3+) to form potassium ferrocyanide (Fe2+), which then reacts with ferric chloride to form ferric-ferrous complex that has an absorption maximum at 700 nm [21].

The ability to reduce Fe3+ may be attributed to hydrogen donation from phenolic compounds which are also related to presence of reducing agents. In addition, the number and position of hydroxyl group of phenolic compounds also plays vital role in their antioxidant activity. However, the action of antioxidants has been allocated to different system such as prevention of chain initiation, binding of transition-metal ion catalysts, decomposition of peroxides, and prevention of continued hydrogen abstraction, reductive capacity and radical scavenging [22].

It has been accepted that flavonoids act as antioxidants through scavenging or chelating process and play significant role in human health and fitness [23]. Antioxidant study was carried out in *Ashokarishta* in in-

vitro models [24] and a high antioxidant potential of *Ashokarishta* was attributed to presence of phenols and flavonoids. Antioxidant assessment study on *Adiantum trapeziforme* clearly indicated that higher antioxidant potential, radical scavenging activity and inhibition of H_2O_2 action on DNA damage was significantly correlated to the presence of phenols, flavonoids, tannins and ascorbic acid present in mature fronds [25].

The results on percentage inhibition of TBRS in lipid peroxidation are given in table 5. The PHF showed a maximum inhibition of TBRS of 60.42 % at 125 µg ml^{-1} whereas for ascorbic acid (standard) was found to be 86.52 % at 125 µg ml^{-1}. The lipid peroxidation IC_{50} values in goat liver model for the PHF and ascorbic acid were found to be 73.87 µg $ml^{-1}$and 32.63 µg ml^{-1} respectively. In the human erythrocyte model the PHF showed a maximum inhibition of TBRS of 75.60 % at 125 µg ml^{-1} as against 38.98 % at 125 µg ml^{-1} of ascorbic acid. The lipid peroxidation IC_{50} values in erythrocyte model for the PHF and ascorbic acid were found to be 72.40 µg $ml^{-1}$and 34.47 µg ml^{-1} respectively. From the results it is seen that hydrogen peroxide treatment in goat liver tissues and erythrocytes resulted in an increase in TBRS. However, the TBRS level was significantly lowered in goat liver cells and human erythrocytes treated PHF.

Phenolic and flavonoids compounds have health applications as they are recognized as potent antioxidants, exerting antioxidative function as terminators of free radicals and chelating metals that are capable of catalysing lipid peroxidation. They may act by donating a hydrogen atom to radicals, which results in the formation relatively stable phenoxy radical intermediates, making it more difficult for a new chain reaction to initiate [26]. Tannic acid was found to be an effective antioxidant and it can be used for minimizing or preventing lipid oxidation in food products, retarding the formation of toxic oxidation products, maintaining nutritional quality and prolonging the shelf life of foods and pharmaceuticals [27]. Both gallic and tannic acids have been considered as a free radical scavengers and consequently as a lipid peroxidation inhibitors. Gallic acid was found to be beneficial in the treatment of myocardial damage associated with type-1 diabetes. It was also reported that gallic acid has a hepatoprotective and antilipid peroxidation in diabetic conditions [28].

Ascorbic acid is a water-soluble antioxidant that acts as the body's primary defense against peroxyl radicals. It is the only antioxidant in plasma which to totally restrains oxidative modification of low density lipoprotein caused due to aqueous peroxyl radicals. Ascorbic acid consumes oxygen free radicals and helps to preserve alpha tocopherol in lipoproteins. A lipid peroxidation study in patients suffering from coronary artery disease showed strong negative relationship between lipid peroxidation and ascorbic acid [29].

The results related to lipid peroxidation determined in terms of MDA levels in human erythrocytes treated with H_2O_2 and PHF are given in table 2. The percent changes in MDA content of different treatments at 30 minutes of incubation were calculated with respect to the control of the corresponding incubation time and was considered as indicator of the extent of lipid peroxidation. The results of present investigation indicate that H_2O_2 is responsible for membrane damage due to lipid peroxidation, which may be the reason for increased MDA level in H_2O_2 treated erythrocytes. However, on the other hand PHF treated erythrocytes showed comparatively less production of MDA, which indicate that antioxidant present in PHF might have reacted with H_2O_2 and minimized its effect on peroxidation, which could be the reason for less production of MDA. Recently a similar antilipid peroxidation effect of polyherbal fermented medicine-Ashokarishta [30] and Khadirarishta [31] was reported in ex-vivo human erythrocyte model. Authors attributed antilipid peroxidation potential of *Ashokarishta* and *Khadirarishta* to the presence of phenols and falvonoids.

The antilipid peroxidation capacity of PHF can be attributed to presence of ascorbic acid, phenols and flavonoids including tannic acid and gallic acid in it. This activity might be due to existence of multiple hydroxyl groups in each phenolic compound which might have donated their protons to break the chain reaction of free radicals [32] and inhibited lipid peroxidation of liver tissues and erythrocyte membranes. In present study we conclude that PHF showed presence of multi-antioxidant compounds which might have synergistically contributed to antioxidant activities and pin down the lipid peroxidation in human erythrocytes and goat liver models.

CONCLUSIONS

Bio-converted PHF exhibited substantial increase in DPPH radical scavenging activity, reducing power, H_2O_2 radical scavenging activity and iron chelation power *in-vitro* models. However, PHF exhibited poor hydroxyl radical and nitric oxide radical scavenging activity as compared to standard ascorbic acid. It also showed more inhibition of lipid peroxidation in the in-vitro human RBC and goat liver models. Antioxidant activity of PHF might be due to presence of phenolic and flavonoids compounds, which are known to have significant antioxidant effects.

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