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RESEARCH ARTICLE

Development and Evaluation of *In-Vitro* Antioxidant Potential and *In-Vivo* Anti-Ageing Activity of Polyherbal Formulation

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ABSTRACT

Aging is indicated by a slow, gradual, structural and functional decline transformation that occurs at various levels of cells, tissues and organs. In human body, oxidative stress play major role in ageing process. Now a day, the free radical theory of ageing can help to understand the process of ageing and search for the effective anti ageing agents. Previous literature review of research has indicated that many of the traditional plants possess potent anti-ageing activity. Present study focuses on different theories of ageing and anti-ageing effects of polyherbal formulation (PHF) of *Emblica officinalis*, *Curcuma longa*, *Tribulus terrestris* and *Asparagus racemosus*. Moreover, the phytochemical characterization and antioxidant potential of the extract was also measured by determining total phenolic contents, DPPH (2,2-diphenyl-β-picrylhydrazyl), reducing power assay which are estimated in in-vitro study. In-vivo anti-ageing activity performed by using D-galactose induced ageing model. Biochemical investigation was done for lipid peroxidation, lipofuscinogensis and total protein. The present study demonstrated that PHF have significant anti ageing capacity, safety and potential to demolish the oxidative stress in body.

KEYWORDS

Polyherbal, Ageing, Oxidative Stress, Antioxidant, DPPH

INTRODUCTION

Ageing has been defined as the progressive loss of function accompanied by decreasing fertility and increasing mortality and disability[1]. Ageing is a physiological state which involves progressive decline of functions of organ and tissues along with development of age-related diseases. The mechanism of ageing process is related to a multifactorial reason. Till date, the free radical and mitochondrial theories reported mechanism on aging. Such theories evidences that oxidative stress within mitochondria is a key factor in producing ageing.

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It results into damage of mitochondria which produces larger amounts of reactive oxygen species i.e., free radicals and leads to progressive damage. The aging process due to oxidative stress can be corrected by environmental, nutritional and pharmacological effective strategies. The free radicals and oxidative stress plays a key role in developing aging in organs such as kidney and human pathological conditions[3].

In recent years the concept of polyherbalism is reported in *Ayurvedic* literature '*Sarangdhar Samhita*' in order to achieve greater therapeutic efficacy. The concept of polyherblism or polypharmacy is based upon use of more than one herbal extract result in enhancement of therapeutic efficacy of many medicinal herbs.

Although, there are well established phytochemical constituent present in extract which are present in less concentration and therefore unable to achieve desired therapeutic effects. On combining constituents of multiple herbs in a particular ratio of concentration, it will synergistically give a better therapeutic efficacy[2]. Because of many advantages of polyherbalism it also gained significant clinical importance.

The synergistic action of combined multiple herb extract attributed to enhancement of pharmacokinetic and pharmacodynamic profile. On the other hand, studies indicated the synergistic effect when active constituents with similar therapeutic activity are targeted to a similar receptor or physiological system. Here, combination of herbal extracts leads to many advantages such as better therapeutic activity, potential pharmacological activity, reduction in herbal dose, reduction in adverse side effects and better patient compliance etc. as compared to single herbal extract[2].

The reported theory of free radical of aging indicates that free radicals are responsible for the age-related disease that occurs at various cellular and tissue levels. In a normal condition there is a balanced-equilibrium occurs between oxidants, antioxidants and bio molecules. The formation of larger amount of free radicals may restrict natural cellular antioxidant defences mechanism result into oxidation and also responsible for defects in cellular functional impairment. The reported data indicates that free radical acts as promoters in aging process. The reduction of free radicals ultimately will result in reduction of ageing changes, aging rate and diseases pathogenesis due to aging.

Antioxidants are inhibitors of oxidation which prevent or delay the oxidation of substance present. Endogenous antioxidant defences are of mainly categorized into two types such as non-enzymatic (e.g., uric acid, glutathione, bilirubin, thiols, albumin, and nutritional factors, including vitamins and phenols) and enzymatic (e.g., the superoxide dismutases, the glutathione peroxidases [GSHPx], and catalase). Antioxidants mainly serve as free radical scavengers by acting in various mechanisms such as a) direct neutralization of free radicals b) reduction of peroxide concentration c) quenching of iron production in order to reduce ROS production d) lipid metabolism such as short chain fatty acid neutralisation of reactive oxygen species[4].

In this present research work we have assessed antiaging activity of polyherbal formulation. Here we have carried out extraction of selected plants of *Asparagus racemosus (*Shatavari), *Emblica officinalis (*Amla), *Curcuma longa* (Turmeric), *and Tribulus terrestris* (Gokhru) using suitable extraction technique. Furthermore, we studied in-vitro antioxidant potential of extract and also investigated the anti ageing potential of polyherbal formulation by D-galactose model.

MATERIAL AND METHODS

Collection of Crude Drug

The powders of fruit of *Emblica officinalis*, *Tribulus terrestris*, roots of *Asparagus racemosus*, *Curcuma longa* was collected from Green Pharmacy, Pune. All other chemical used of analytical grade.

Extraction Procedures

Emblica officinalis

The powder was extracted with distilled water in soxhlet apparatus for 36 hours. The extract is filtered and vaccum evaporated[5].

Tribulus terrestris

The powder was extracted with distilled water in soxhlet apparatus for 36 hours. The extract is filtered and vaccum evaporated[6].

Curcuma longa

For the extraction, the rhizome of C. longa was macerated with hot water (80°C) for 4 h and the aqueous extract was evaporated under vacuum at 60°C. The rhizome residue was re-extracted with ethanol at 60°C for 2 h, filtered and evaporated under vacuum. The final extract was a 1:1 mixture of the aqueous extract and the alcoholic extract, are dissolved with water and alcohol, respectively[7].

Asparagus racemosus

The powder was extracted with distilled water in soxhlet apparatus for 36 hours. The extract is filtered and vaccum evaporated.

Preparation of Polyherbal Formulation [PHF]

Table: 1 Proportion of Extracts in Polyherbal Formulation

Proportions of plant in Polyherbal formulation: Name of Plant Extracts	Proportion
Emblica officinalis	25%
Tribulus terrestris	25%
Curcuma longa	25%
Asparagus racemosus	25%

***In-Vitro* Antioxidant Potential of PHF**

***Total Phenol Content*[8]**

Total phenolic content (TPC) of polyherbal extract *was* estimated by the literature method of Folin-Ciocalteu reaction. Gallic acid was used as standard (Sigma Aldrich Company, USA). To 100 µL of extract (100 µg/mL), add 500 µL of (50%) Folin-Ciocalteu reagent and flask was shaken thoroughly. It is then followed by subsequent addition of 1 mL of 20% Na_2CO_3 solution. A mixture was then incubated at room temperature for 20 min .The absorbance was measured at 730 nm. The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per gram samples.

Determination of 1, 1-diphenyl-2-picryl hydrazyl (DPPH) Radical Scavenging Activity

The free radical scavenging activity of PHF and Ascorbic acid was measured in terms of hydrogen donating ability or radical scavenging ability by means of the stable radical DPPH. 0.1 mM solution of DPPH in ethanol was prepared and 1.0 ml of this solution was added to 3.0 ml of extract solution in water at different concentrations (100- g/ml). After 30 min. the absorbance was measured at 517 nm. Lower absorbance values of the reaction mixture indicate higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation:

Where, A control = Absorbance of control reaction

A test = Absorbance of test reaction

% free radical scavenging activity = [(A control – A sample)] / A control

Where, A is the absorbance.

Determination of Reducing Power

The reducing power of the extracts was determined according to the method of Oyaizu (1986). Various concentrations of the extract (100-500 mg/ml) in 1.0 ml of deionized water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and 1% potassium ferricyanide (2.5 ml). The mixture incubated at 500 C for 20 min. Aliquots of TCA (2.5 ml), 10% were added to the mixture, which was centrifuged at 1036 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and freshly prepared $FeCl_3$ solution (0.5 ml, 0.1%). The absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increase reducing power

***In-Vivo* Evaluation of Anti-ageing activity[9, 10]**

Specification of Animals and Extracts

A. Animal Used

Five months old male mice weighing about 40 gm were housed under standard laboratory conditions in 12hrs light- dark cycle. Animals were fed on standard diet and water ad libitum. The protocol for experimentation was approved by Institutional Animal Ethics Committee (IAEC) of Poona College of Pharmacy, Pune, India (CPCSEA/04/2012) Constituted under Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA), India (CPCSEA/100/1999).

B. Preparation of Extracts

The prepared herbal composition in the form of aqueous suspension were given to the animals as test drug with dose 200mg/kg; p.o. and 400 mg/kg; p.o. The solution was kept in airtight,

amber colored bottle and stored at room temperature until ready for use.

C. Volume of Drug Administration

The volume of drug solution to be administered was calculated based upon the body weight of animals.

D. Route of Administration

The PHF was administered through per oral (p.o.) route. The D-galactose was administered through subcutaneous route (s.c.)

In- vivo Antiageing Activity

The mice were divided into following eight groups of six animal each (n=6)

Group I:- Control

No drug, no 5% D-galactose.

Group II:- Ageing induced

0.5 ml of 5% D- galactose was injected (s.c.) / animal/day for 30 days.

Group III:- Standard

0.5 ml of 5% D- galactose was injected (s.c.) and 0.5ml Triphala churn (p.o.) is administered per animal/day for 30 days.

Group IV:- Lower dose(100mg/kg)

0.5 ml of 5% D- galactose was injected (s.c.) and 0.5 ml PHF (p.o.) is administered per animal/ day for 30 days.

Group V:- Middle dose (200mg/kg)

0.5 ml of 5% D- galactose was injected (s.c.) and 0.5 ml PHF (p.o.) is administered per animal/ day for 30 days.

Group VI:- Higher dose (400mg/kg)

0.5 ml of 5% D- galactose was injected (s.c.) and 0.5 ml PHF (p.o.) is administered per animal/ day for 30 days.

The change in body weight was recorded daily. All the animals were sacrificed on the next day of last treatment by cervical dislocation. Different organs like kidney and liver were dissected out and weighed and used for following investigation:

A. Lipid peroxidation

B. Lipofuscinogenesis

C. Total protein content

A. Anti-Lipid Per oxidation (Melanodialdehyde)[11, 12]

Decomposition of lipid in the body leads to the formation of Malondialdehyde (MDA) along with other aldehydes and enals as the end product. These react with thiobarbituric acid to form colored complexes. Hence these are called as the Thiobarbituric acid reactive substances (TBARS). The complex of TBA-MDA is selectively detected at 532 nm using UV spectrophotometer.

B. Anti-Lipofuscinogenesis[13]

Procedure

Tissue was homogenized by using mixture prepared earlier for lipid per oxidation. The extraction is carried out by addition of chloroform: methanol (2:1 v/v) to 0.5 ml tissue homogenized sample. It was mixed well on vortex mixer and then 3ml of double distilled water was added and centrifuged at 300 g for 2 min. To 1ml upper layer 0.1ml methanol was added and fluorescence was measured on photoflurometer calibrated with quinine sulphate. 1µg of quinine sulphate/ml of 0.1 N H_2SO_4 was used as standard and 0.1N H_2SO_4 was used as blank.

C. Determination of Total Protein Content

The total protein was estimated by Lowry's method. The method is based on both the biuret reaction where the peptide bonds of proteins react with copper under alkaline conditions produces Cu+, which reacts with the Folin reagent and the Folin–Ciocalteau reaction, phosphomolybdotungstate is reduced to heteopolymoybdenum blue by the copper-catalyzed oxidation of aromatic amino acids. The reactions result in a strong blue color, which depends partly on the tyrosine and tryptophan content. Total protein reacts with cupric ions in an alkaline medium to form a bluish – violet colored complex. The intensity of the color formed is directly proportional to the amount of

Total Protein present in the sample. The reaction is carried out at 550 nm wavelength.

RESULTS AND DISCUSSION

Aging is complex and multifactorial phenomenon that results into physiological functional decline which usually manifest after maturity and responsible for disability and death. The various biological and cellular mechanisms are involved in ageing. According to Fontana and Klein oxidative stress–induced protein and DNA damage is one of the mechanisms involved in ageing process[14].

The antioxidant is "any substance, when present at low concentrations compared with that of an oxidizable substrate that significantly delays or prevents oxidations of that substrate". The term oxidizable substrate includes every type of molecule found in vivo. Antioxidant defense include the antioxidant enzymes like SOD, CAT, GSH-px, etc, low molecular agents and dietary antioxidants.

In ancient times, Ayurvedic physicians had established certain dietary and therapeutic measures in order to arrest/delay ageing in order to rejuvenate whole functional dynamics of the body system. This revitalization as well as rejuvenation is termed as the "Rasayana chikitsa" (rejuvenation therapy). These Rasayana plants are reported to have the following characteristics: they result into prevention of ageing, re-establishment of youth, strengthening of life, improvement of brain power and prevention of diseases, all of which collectively responsible for increase in the resistance of the human body against various diseases. According Shushruta "Rasayana" therapy says that it arrests ageing ("Vayasthapam"), increase life span, intelligence and strength and therefore responsible one to prevent disease[15].

For the present study, four different indigenous plants were selected namely,

1. *Emblica officinalis:* - Amla
2. *Curcuma longa:* - Turmeric
3. *Tribulus terrestris:* - Gokhru
4. *Asparagus racemosus:* - Shatavari

Here in this study ,the total phenolic content of polyherbal extract was found to be 450 mg GAE/100gm of sample .The linear equation y=176.95x – 0.0204 for the calibration curve of gallic acid .The Antioxidant parameter has been directly connected to the presence of phenolic content present in the molecular structure of many of natural antioxidants. Phenolic compounds are mainly occurring as secondary metabolites which are synthesized by plants. They indicate various biological properties such as: antioxidant, anti-apoptosis, anti-aging, anti-cancer, anti-inflammatory, anti-atherosclerosis, cardiovascular protection, improvement of the endothelial function, as well as inhibition of angiogenesis and cell proliferation activity etc. Many of these biological actions are occurs due to their intrinsic reducing potential and radicals scavenging properties[16,17]. The hydrogen donating characteristics of polyphenolic compounds is main reson for inhibition of free radical induced lipid peroxidation[18].

Table 2: Determination of 1, 1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity

Group	Concentration (µg/ml)	% Inhibition
PHF	20	40.55
	40	50.98
	60	70.21
	100	91.11
Ascorbic acid	20	45.78
	40	59.9
	60	80.32
	80	90.32
	100	96.34

"Rasayana" leads to their therapeutic actions by owing their ability to scavenge free radicals or by possessing antioxidant potential. Ayurveda literally also termed as 'science of life and longevity' as it contains several plants which have been deals with ageing treatments. At the concentration of 100 mg/ml extract, PHF inhibits 91.11% of DPPH radical. The reducing ability of a compound may serve as a significant indicator of its potential antioxidant. Reducing ability of the extract increased with increasing amount of sample. At concentration 500 mg/mL extract shows significant activity.

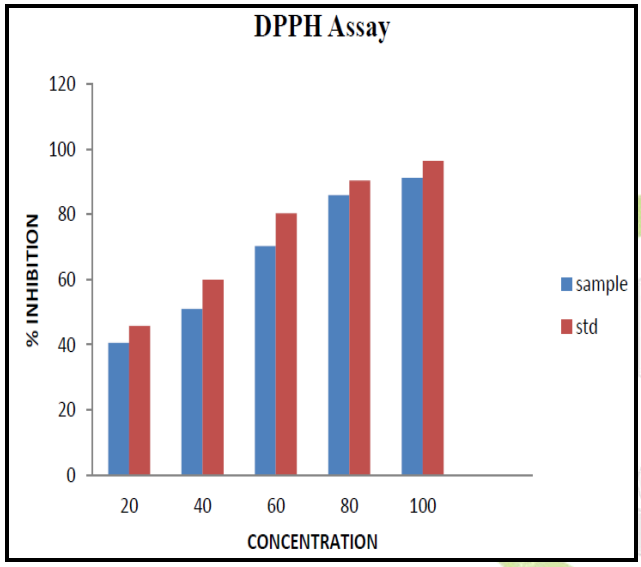

Figure 1: Graph of DPPH Free Radical Scavenging Activity

The DPPH antioxidant assay is based on the capability of DPPH, a stable free radical, to decolorize in the presence of antioxidants. The antioxidant reacts with DPPH radical (purple color) and converts it into a colourless a-a-diphenyl-b- picryl hydrazide. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance. PHF shows effective antioxidant potential on increasing concentration. At the concentration of 100 mg/ml extract, PHF inhibits 91.11% of DPPH radical. The reducing ability of a compound may serve as a significant indicator of its potential antioxidant. At the concentration of 100 mg/ml extract; this inhibits 91.11% of DPPH radical.

Table 3: Determination of reducing power

Group	Concentration (µg/ml)	% Inhibition
PHF	100	0.0132
	200	0.057
	300	0.089
	400	0.123
	500	0.15
Ascorbic acid	100	0.0148
	200	0.0678
	300	0.099
	400	0.129
	500	0.159

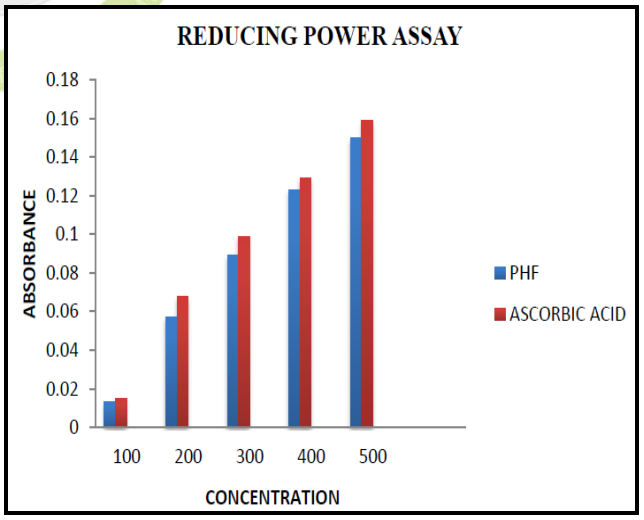

Figure 2: Graph of Determination of Reducing Power Assay

This method is based on the basic principle of increase in the absorbance of the reaction mixture components. Increase in the absorbance indicates that there is increase in the antioxidant activity.

In this method antioxidant compound forms a colored complex with potassium ferricyanide, trichloro acetic acid and ferric chloride, which can be measured at 700 nm. The reducing ability of the compound is a significant indicator of its antioxidant potential and radical scavenging property. Here reducing ability of the polyherbal extract increased with increasing amount of sample. At concentration 500 mg/mL extract shows significant activity. Reducing ability of the extract increased with increasing amount of sample. At concentration 500 mg/mL extract shows significant activity. *Emblica officinalis* is rich in Vit.C it also contain Emblicanin A & B, pedunculagin, punigluconin which act as antioxidants that catalytically remove free radicals and other reactive species like SOD, CAT, peroxidase and thio. (saponins) which increases fertility, in male increases libido, in female it potentiates oestrus and increases fertility.

Curcuma longa contains curcumin, volatile oil, resins which decreases lipofuscin granules and decrease in fluorescence and it act as antiageing and antiwrinkle properties. *Tribulus terrestris* contains Protodioscin Diosgenin, Gitogenin

Asparagus racemosus contains shatavarin I – IV, rutin, Quercetin, diosgenin, which shows antioxidant activity through the free radical scavenging. All these properties results in significant antioxidant potential synergistically. In *in-vivo* anti ageing activity study, the lipofuscin (age pigment) accumulates slowly, universally & specifically in lysosomes. The composition of lipofuscin is nearly half protein, one-third carbohydrate & rest of lipid indicates that it is primarily composed of advanced glycation end product (AGE) rather than lipid peroxidation product. Oxidative stress has been shown to promote lipofuscin formation whereas antioxidant reduced it.

Determination of % Inhibition of MDA

Table 4: Effect of Dg and formulation in liver and kidney homogenate

Effect of Dg & formulation in liver & kidney	**Control**	**Ageing Induced**	**Standard**	**Lower dose (100mg/kg)**	**Middle dose (200 mg/kg)**	**Higher dose (400mg/kg)**
% Inhibition of MDA in male liver	30.87±5.28	26.94±3.86	60.05±6.08	38.25±3.14	45.03±2.29	55.84±5.36
% Inhibition of MDA in female liver	20.42±1.40	15.76±1.54	87.75±1.61	24.57±1.30	39.62±2.35	83.56±1.93
% Inhibition of MDA in male kidney	19.39±2.06	16.09±2.03	81.94±2.92	24.52±5.11	37.67±3.68	77.00±3.09
% Inhibition of MDA in female kidney	26.18±1.50	23.36±1.78	81.55±1.52	28.46±1.45	33.98±2.16	81.31±0.91

In- vivo antiageing activity A. Lipid peroxidation B. Lipofuscinogenesis C. Total protein content was determined. In anti-Lipid Peroxidation (Melano dialdehyde) increased malondialdehyde is an indication of increased lipid peroxidation in D-galactose treated mice this is due to increased oxidative stress and there is positive correlation between lipid peroxidation and oxidative stress.

D-galactose is known to accelerate the process of ageing by formation of advanced glycation end products (AGEs).

D-galactose is a reducing sugar, which reacts with free amino group of proteins, amino acids to form insoluble aggregates called as Advanced Glycation End Products.

The later produces fifty folds more free radicals than non glycated proteins.

These free radicals are superoxide radicals generated by protein bound Amadori products in the presence of transition metal ions such as iron. The superoxide radicals are dismutated to hydrogen peroxide and results in the generation of lethal hydroxyl radicals. AGEs can increase the oxidative stress by:

1) Modulating cellular functions by binding to specific cell surface receptor molecule called RAGE (Receptor for Advanced Glycation End products) and

2) Activate the intercellular signal transduction mechanisms and evoke formation of free radicals the reaction is carried out at 532nm.

MDA increases in male mice than in female mice which indicate that ageing is more in male mice than in female mice. Higher and middle dose are significant.

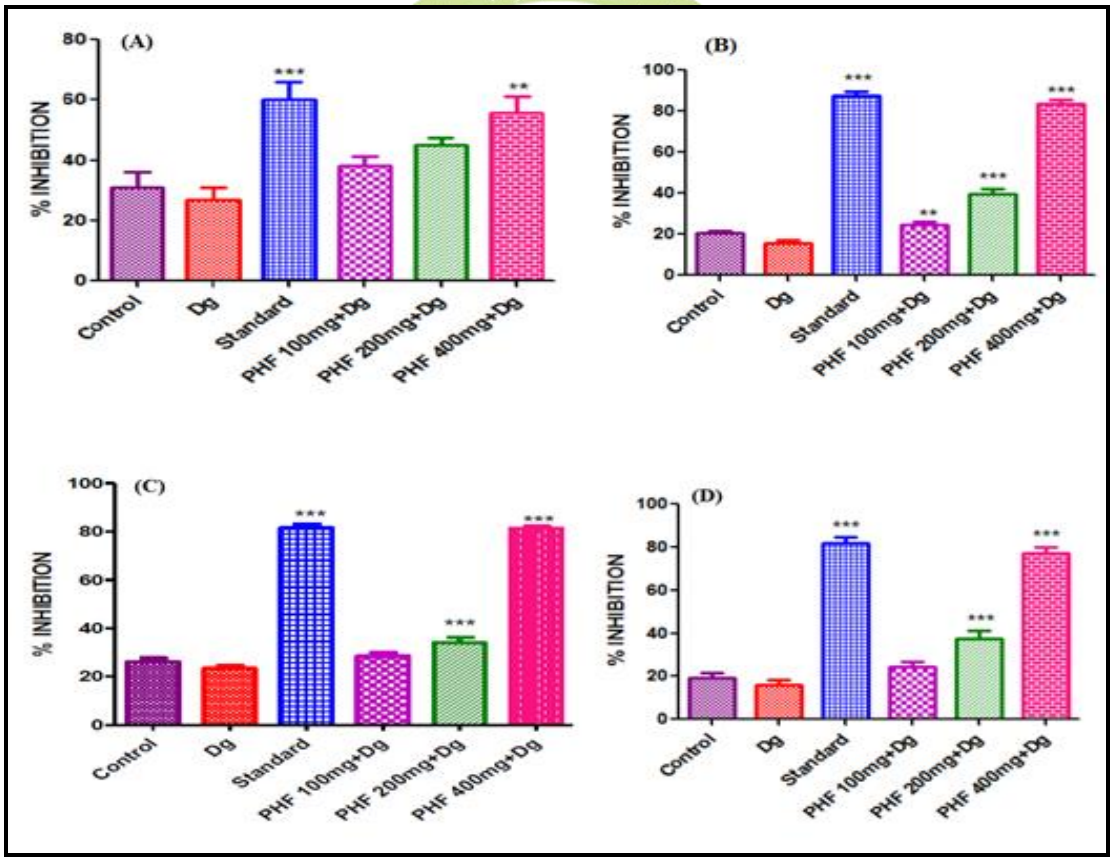

Figure: 3 (A) Graph of Anti- Lipid Peroxidation in Male Liver (B) Graph of Anti- Lipid Peroxidation in Female Liver (C) Graph of Anti- Lipid Peroxidation in Male Kidney(D) Graph of Anti- Lipid Peroxidation in Female Kidney. Data was Analyzed by One-Way ANOVA Followed by Dunnett's Post-Test Test Using Graph Pad Instat Software; P Value (*P<0.05,**P<0.01,***P<0.001)

Determination of Lipofuscinogenesis

Table 5: Effect of Dg and Formulation in Liver and Kideny

Effect of Dg and formulation in liver and kideny	Control	Ageing induced	Standard	Lower dose (100mg/kg)	Middle dose (200 mg/kg)	Higher dose (400mg/kg)
Intensity of fluroscence in male liver	4.77±0.29	7.46±0.41	2.48±0.38	6.36±0.48	4.47±0.20	3.56±0.35
Intensity of fluroscence in female liver	5.27±0.29	7.52±0.30	2.57±0.17	5.60±0.29	4.23±0.16	2.55±0.18
Intensity of fluroscence in male kidney	4.03±0.41	7.22±0.38	3.57±0.34	6.43±0.39	4.86±0.46	2.95±0.49
Intensity of fluroscence in female kidney	4.46±0.66	7.87±0.35	3.50±0.46	6.69±0.39	5.15±0.22	3.94±0.28

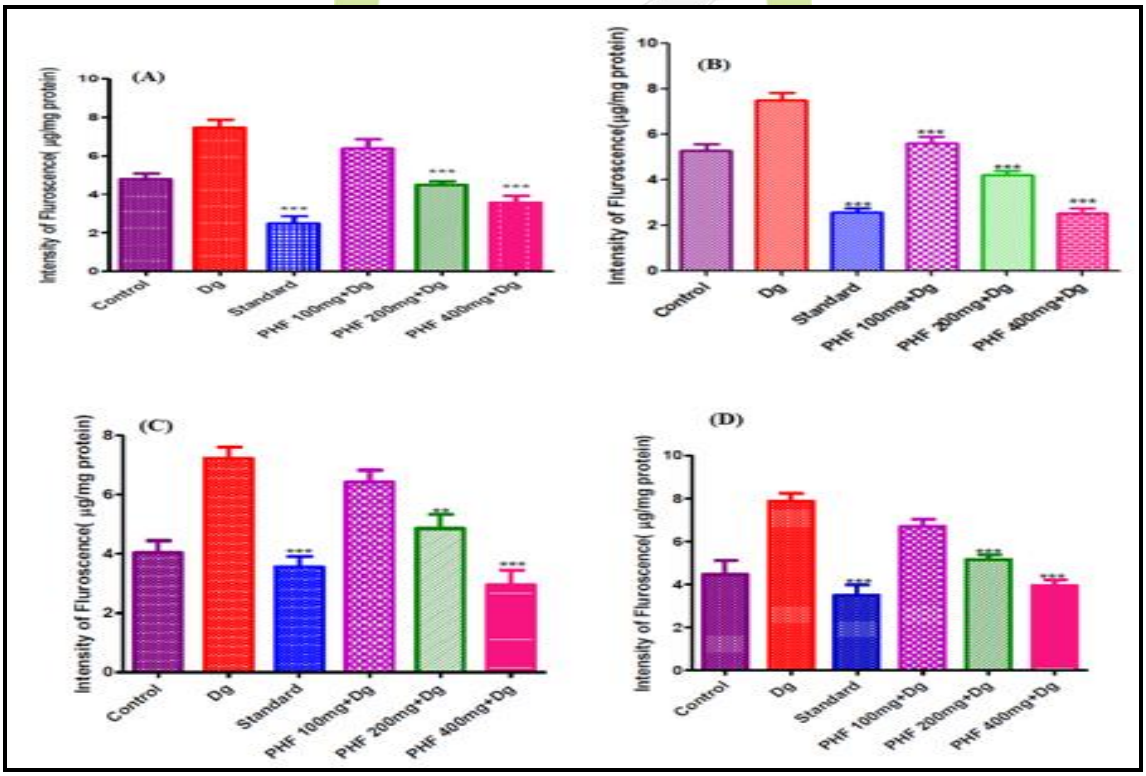

Figure: 4 (A) Intensity of Fluorescence in Male Liver (B) Intensity of Fluorescence in Female Liver (C) Intensity of Fluorescence in Male Kidney (D) Intensity of Fluorescence in Female Kidney. Data was analyzed by One-Way ANOVA Followed by Dunnett's Post-test Test Using Graph Pad Instat Software; P Value (*P<0.05, **P<0.01,***P<0.001)

The intensity of fluorescence indicated that lipofuscinogenesis was increased in Ageing induced mice than the control. The fluorescence was measured on spectrofluorometer at 366 excitation spectrum. In this investigation it is observe that ageing induced group shows increased lipofuscinogenesis than control. It shows that male mice shows more lipofuscinogenesis than the female mice which indicates that ageing is more in male mice as compared in female mice.

Total protein reacts with cupric ions in an alkaline medium to form a bluish – violet colored complex. The intensity of the color formed is directly proportional to the amount of Total Protein present in the sample. The reaction is carried out at 550 nm wavelength.

Total protein in gm/dl= (Abs T) – (Abs. B) / (Abs. S)-(Abs.B)

The total protein content in male mice serum is more than in female mice. Increased malondialdehyde is an indication of increased lipid peroxidation in D-galactose treated mice this is due to increased oxidative stress and there is positive correlation between lipid peroxidation and oxidative stress.

D-galactose is a reducing sugar, which reacts with free amino group of proteins, amino acids to form insoluble aggregates called as Advanced Glycation End Products. The reaction is carried out at 532nm. MDA increases in male mice than in female mice which indicate that ageing is more in male mice than in female mice. Higher and middle dose are significant. The intensity of fluorescence indicated that Lipofuscinogenesis was increased in ageing induced mice than the control.

The fluorescence was measured on spectrofluorometer at 366 excitation spectrum. In this investigation it is observe that ageing induced group shows increased Lipofuscinogenesis than control. It shows that male mice shows more Lipofuscinogenesis than the female mice which indicates that ageing is more in male mice as compared in female mice. Total protein reacts with cupric ions in an alkaline medium to form a bluish – violet coloured complex. The intensity of the colour formed is directly proportional to the amount of Total Protein present in the sample. The reaction is carried out at 550 nm wavelength. The total protein content in male mice serum is more than in female mice.

Table 6: Total Protein Content in Serum

Total Protein Content in Serum	**Control**	**Ageing induced**	**Standard**	**Lower dose (100mg/kg)**	**Middle dose (200 mg/kg)**	**Higher dose (400mg/kg)**
Total protein content in male serum	0.94± 0.17	0.79± 0.20	2.45± 0.16	0.34± 0.09	1.26± 0.09	1.68± 0.16
Total protein content in female serum	0.32± 0.07	0.37± 0.09	2.50± 0.12	0.34± 0.08	1.19± 0.11	1.50± 0.14

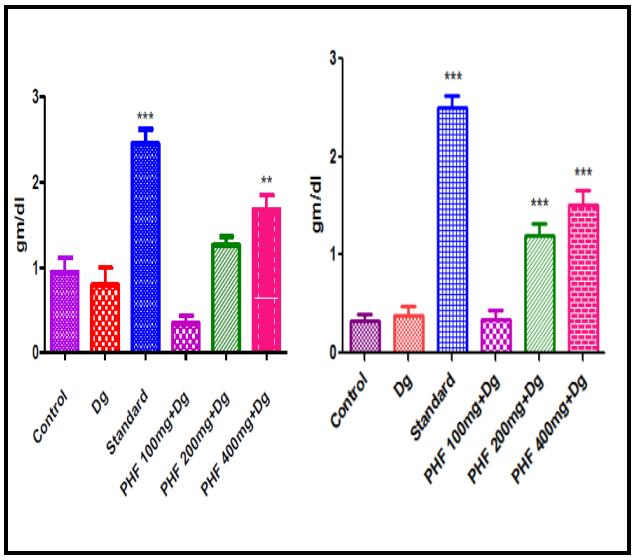

Figure: 5 (A) Graph of Total Protein Content in Male Serum (B) Graph of Total Protein Content in Female Serum. Data was analyzed by One-way ANOVA Followed by Dunnett's Post-test Using Graph pad Instat Software; P Value (*P<0.05,**P<0.01,***P<0.001)

CONCLUSION

In the present study the formulation is screen against anti-ageing activity using anti-ageing model namely D-galactose induced ageing model, formulation showed decrease in ageing at 200 mg/kg and 400 mg/kg, however effect at 400 mg/kg was more pronounced. All the extracts also showed an effective *in-vitro* antioxidant activity. The ageing process is now major risk factor for disease & death in the developed countries and population of world is ageing rapidly. Future significant increase in average life expectancy i.e. functional life expectancy will be achieved only by slowing rate of production of ageing changes by ageing process. Thus there is need to develop potent herbal anti-ageing formulation without interactions. Also formulation is a mixture of four herbs therefore results cannot be conclusive without herb-herb drug interaction data, which we aim to explore in future.

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