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PRELIMINARY PHYTOCHEMICAL SCREENING AND *IN VITRO* ANTIOXIDANT ACTIVITY OF METHANOL ETHANOL AND AQUEOUS EXTRACT OF POLYHERBAL FORMULATION (PHF)

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ABSTRACT
Preliminary phytochemicals screening is the most valuable step, in the detection of the bioactive compounds present in medicinal plants and successively may lead to the discovery and development of new drugs. In the present study, three different extracts namely ethanol, methanol and aqueous of polyherbal formulation were screened for their phytochemical composition and *in vitro* antioxidant activity in order to relate their presence with bioactivities of the plants. Phytochemical screening tests was conducted for polyherbal formulation and found that extracts contains a variety of phytochemicals like saponins, tannins, flavonoids, terpenoids, glycosides and reducing sugars and among which there is higher level of precipitation for phenol and flavonoids. The free radical scavenging activity of the extract were determined by scavenging 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH), lipid peroxidation (LPO), nitric oxide (NO), superoxide (SOD) and hydroxyl radical scavenging activities (H_2O_2), reducing power and total antioxidant. compared to ethanolic and aqueous extract, methanolic extract of polyherbal formulation has a higher percentage of inhibition of DPPH radical scavenging activity (84.14%), NO scavenging activity (81.16%), superoxide and (80.68%), hydroxyl radical (75.25%), LPO (79.35%), total antioxidant activity (48 µg α-tocopherol/g) and reducing power (34 µg of ascorbic acid/g). Hence the present studies indicated that the methanolic extract held higher radical scavenging activities, reducing power, total antioxidant activity with higher levels of total flavonoids and total phenols than the ethanol and aqueous extract of polyherbal formulation.

KEYWORDS: Medicinal plant, Polyherbal formulation, Phytochemical, *in vitro* antioxidant.

INTRODUCTION
Natural products particularly from plant sources, including species are the known complementary and alternative medicine systems throughout the world and continued to deliver mankind with new medications.[1] Medicinal plants created huge demand in food, cosmetics, and pharmaceutical industries associated with their physicochemical properties and biological activities. Almost 80% of individual's use traditional medicine in the developed countries, which has compounds derived from medicinal plants. Traditional products from the medicinal plants provide unlimited prospects for the development of new drugs and shown to have favourable therapeutic potential.[2] Our body cells produce free radicals whenever they use oxygen which will cause damage to cells, various scientists has confirmed that antioxidants are the most effective tools to eliminate free radicals, which cause oxidative stress and are possible defensive agents that protect the cells from reactive oxygen spices.[3,4] Phytochemical component and secondary metabolites defines the medicinal value of a plant, including: total phenol, flavonoids, alkaloids, tannins, saponin, glycosides and other stress gene response products.[5] The usage of drugs in day to day life is too expensive and therefore the search for some cheap sources from plant substances in nature become inevitable for the majority of the population. The side effects of synthetic anti-inflammatory drugs including gastric injury, ulceration, bronchospasm, inhibition of platelet aggregation, liver and kidney toxicity, have limited their use.[6] Therefore, it is mandatory to investigate for new anti-inflammatory drugs with fewer side effects and cost effectiveness.[7] In this present era the assurance for the standardization of medicinal plants in terms of safety, quality and efficacy has become an important issue. It turn out to be extremely essential to make an effort towards standardization of the plant material used for therapeutic purposes.[8]

The combination of several plant ingredients when used in single formulation, it enrich the beneficial effects

through synergistic amplification, diminishes any possible adverse effects and offers advantage over a single isolated ingredient.[9] Hence, in the present study, Polyherbal formulation, a phytochemical combination constituting flowers of *Cassia auriculata*, *leaves of Gymnema sylvestre*, *seeds of Trigonella foenum graecum*, bark of *Cinnamomum zeylanicum* and seeds of *Syzygium cumini* were mixed in a defined ratio and combined as a single dose and evaluated for the view of phytochemical and *in vitro* antioxidant activity.

S.No.	Plant Name	Common Name	Part Used	Family
1	Cassia Auriculata	Senna auriculata	Flower	Fabaceae
2	Gymnema sylvestre	Gymnema	Leaves	Apocynaceae
3	Trigonella foenum Graecum	Fenugreek	Seeds	Fabaceae
4	Cinnamomum zeylanicum	Cinnamon	Bark	Laurels
5	Syzygium cumini	Jamun	Seeds	Myrtaceae

MATERIALS AND METHODS

The flower of *Cassia auriculata* and leaves of *Gymnema sylvestre* were collected from a local garden in the southern part of India (Kanchipuram and Villupuram District, TamilNadu). Seeds of *Trigonella foenum graecum* (Fenugreek), bark of *Cinnamomum Zeylanicum* and seeds of *Syzygium cumini* were purchased from the market at the commercially matured stage and the plant materials were air-dried under the shade and made into fine power using cutting mill and mixed with the defined ratio.[10]

Extraction Procedure

Methanol, ethanol and aqueous extracts were selected because they have been reported to be the best solvents for the extraction of antioxidant compounds. 50 grams of the extract was weighed accurately and soaked in 100 ml of the three different solvents separately and kept in a dark place for 3 days in a shaker. Carbon dioxide was released frequently. After 3 days, samples were filtered and the filtrates were kept in a water bath at about 40^0C in order to concentrate them. The concentrated filtrates obtained were used for further studies at different concentrations.

Preliminary Phytochemical Screening

The methanolic, ethanolic and aqueous extracts of polyherbal formulation were used to test the preliminary qualitative phytochemical screening were carried out for steroids (Salkowski test), alkaloid (Wagner's Test), flavonoids (Alkaline reagent test, H_2SO_4 test and Lead acetate test), Tannins and phenols (Lead acetate test and Braymer's test), Saponins (Forthing test), Glycosides (Keller-Kiliani test), carbohydrates (Molisch's, Benedict's test and Fehling's Test), following the standard protocols.

Determination of DPPH Radical Scavenging Activity

The free radical scavenging activity of methanolic, ethanolic and aqueous extract of polyherbal formulation was measured by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method of Blois (1958).[11] 0.2mM solution of DPPH in all three solvents was prepared and 100µl of this solution was added to various concentrations of methanolic, ethanolic and aqueous extracts at the concentrations of 10, 20, 40, 60, 80, 100µg/ml. After 30 minutes, absorbance was measured at 517nm. Butylated hydroxytoluene (BHT) was used as the reference material. The IC_{50} values were determined as the concentration of the test mixture that gave 50% reduction in the absorbance from a control blank. All the tests were performed in triplicates and the results were averaged.

% inhibition =

(Absorbance of control - Absorbance of test sample) / Absorbance of control * 100

Determination of Lipid Peroxidation Inhibition Assay

For liver homogenate 10% (chicken liver homogenate purchase from local butcher shop) was prepared using ice-cold potassium chloride (0.15 M) in a Teflon tissue homogenizer and the protein content was adjusted to (500 mg ml^{-1}).[12] In the control system 1ml of tissue homogenate by the addition of ascorbate (100 mM), ferrous sulphate (25 mM), and potassium dihydrogen phosphate (10 mM) the lipid peroxidation was initiated and the volume was made up to 3ml with distilled water and incubated at room temperature for 30 min. homogenate was incubated with various concentrations 10, 20, 40, 60, 80, 100 µg/ml. The inhibition of lipid peroxidation was assessed by the estimation of thiobarbutric acid reactive substances (TBARS) level by measuring the absorbance at 532 nm.[10] Butylated hydroxyl toluene (BHT) used as positive control. The IC_{50} values were determined as the concentration of the test mixture that gave 50% reduction in the absorbance from a control blank. All the tests were performed in triplicates and the results were averaged.

% inhibition =

(Absorbance of control - Absorbance of test sample) / Absorbance of control * 100

Determination of Nitric Oxide Radical Scavenging Activity

Nitric oxide scavenging activity can be estimated by the use of Griess IlIosvoy reaction.[13] NO reacts with oxygen to produce stable products (nitrate and nitrite) under aerobic conditions. The quantities of which can be determined using Griess reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitrite ions. For the experiment, sodium nitroprusside (10mM) in phosphate buffered saline was prepared with different concentrations 10, 20, 40, 60, 80, 100 µg/ml of methanol, ethanol and aqueous extract of polyherbal formulation and incubated at 37^0C for 2

hours. The same reaction mixture without the extract served as the control. After the incubation period, 0.5 ml of Griess reagent (1% sulfanilamide, 2% H_3P0_4 and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride) was added. The absorbance of the chromophore that formed during diazotization of the nitrite with sulfanilamide and subsequent coupling with Naphthylethylenediamine dihydrochloride was immediately read at 550 nm. Inhibition of nitrite formation by the extracts and the standard Butylated hydroxyl toluene (BHT) were calculated using the formula as mentioned earlier IC_{50} which is an inhibitory concentration of each extract required to reduce 50% of the nitric oxide formation was determined. All the tests were performed in triplicates and the results were averaged.

% inhibition =

(Absorbance of control - Absorbance of test sample) / Absorbance of control * 100

Determination of Superoxide Anion Radical Scavenging Activity

The superoxide anion scavenging activity was described by Srinivasan *et al* 2007.[14] The superoxide anion radicals were generated in 3.0 mL of Tris – HCL buffer (16 mM, pH 8.0), containing 0.5 mL of NBT (0.3mM), 0.5 mL NADH (0.936mM) solution, 1.0 mL extract of different concentration (10, 20, 40, 60, 80, 100 µg/ml) and 0.5 mL Tris – HCl buffer (16mM, PH 8.0). The reaction was started by adding 0.5 mL PMS solution (0.12mM) to the mixture, incubated at 25^0C for 5 min and the absorbance was measured at 560 nm against a blank sample, ascorbic acid. Butylated hydroxyl toluene were used as positive control. The IC_{50} values were determined as the concentration of the test mixture that gave 50% reduction in the absorbance from a control blank. All the tests were performed in triplicates and the results were averaged. The percentage inhibition was calculated by using the following equation.

% inhibition =

(Absorbance of control - Absorbance of test sample) / Absorbance of control * 100

Determination of Hydroxyl Radical Scavenging Activity

The scavenging capacity for hydroxyl radical was measured according to the modified method of Halliwell, 1987.[15] Stock solutions of EDTA (1mM), FeCl3 (10mM), Ascorbic Acid (1mM), H_2O_2 (10mM) and Deoxyribose (10 mM), were prepared in distilled deionized water. The assay was performed by adding 0.1ml EDTA, 0.01 ml of FeCl3**,** 0.1mL H_2O_2, 0.36ml of deoxyribose, 1ml of the extract of different concentration (10, 20, 40, 60, 80, 100µg/ml) dissolved in distilled water, 0.33ml of phosphate buffer (50mM , pH 7.9), Butylatedhydroxytoluene (BHT) was used as positive control. The mixture was then incubated at 37^0C for 1 hour. 1ml portion of the incubated mixture was mixed with 1ml of 10% TCA and 1ml of 0.5% TBA (in 0.025M NaOH containing 0.025% BHA) to develop the pink chromogen measured at 532nm. The IC_{50} values were determined as the concentration of the test mixture that gave 50% reduction in the absorbance from a control blank. All the tests were performed in triplicates and the results were averaged.

% inhibition =

(Absorbance of control - Absorbance of test sample) / Absorbance of control * 100

Determination of Total Antioxidant Activity (Tta)

An aliquot of 0.1 ml of Polyherbal formulation was combined with 1 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) in an Eppendroff tube. The tubes were capped, incubated in a water bath at 95°C for 90 min, cooled to room temperature, and the absorbance of each solution was measured at 695 nm against a blank solution.[16]

Determination of Reducing Power

The reducing power of extracts was determined according to the method of Siddhuraju and becker, 2003.[17] The extract was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferric cyanide ($K_3Fe(CN)6$) (2.5 ml, 1%). The mixture was incubated at 50^0C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture to stop the reaction, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and ferric chloride (FeCl3) (0.5 ml, 0.1%), the absorbance was measured at 700 nm. Absorbance increased in the reaction mixture indicated increased reducing power.

Total Phenolic Content

Phenolic contents were estimated by the method described by Singleton and Rossi[18] with some modifications. 1 ml of sample was mixed with 1 ml of Folin–Ciocalteau's phenol reagent; after 3 min, add 1 ml 35% Na_2CO_3, and then add distilled water to make the reaction system reach 10 ml. The reaction mixture was mixed carefully and allowed to stand for 90 min at room temperature in dark place. Absorbance of all the sample solutions was measured at 725 nm against a blank using the spectrophotometer. Total phenolic contents were expressed as milligram of gallic acid. With different concentrations of gallic acid, standard column was constructed.

Total Flavonoid Content

Total flavonoids were measured by the method of Jia, 1999.[19] 1 ml of the sample was put into 10ml volumetric flask containing 4 ml of distilled water; 0.3 ml of 5% $NaNO_2$ was added. After 6 min, 0.3 ml of 10% $Al(NO_3)3$ was added; after 6 min, 2 ml of 1 M NaOH were added. Then to volume with the addition of 2.4 ml of distilled water volumetric flask was diluted immediately and the contents thoroughly mixed. Absorbance of all the sample solutions was measured at 510 nm against a blank using the spectrophotometer. Total flavonoid contents were expressed as milligram of

rutin equivalents. With different concentrations of rutin as the standard column was constructed.

RESULT AND DISCUSSION

Different concentrations of the methanolic, ethanolic, and aqueous extract of polyherbal formulation ranging from 10 to 100 µg/ml were tested for their antioxidant activity using altered *in vitro* models. It was detected that free radicals were scavenged by the test compounds in a concentration dependent manner in all the models.

PHYTOCHEMICAL SCREENING

Phytochemical screening of the methanolic, ethanolic and aqueous extracts revealed the presence of alkaloids, tannins, flavonoids, carbohydrates, phenols, saponins, steroids and glycosides. The results are depicted in **Table 1.**

Table 1: Preliminary phytochemical screening of Polyherbal formulation (PHF).

Phytochemical compounds	Methanolic extract of PHF	Ethanolic extract of PHF	Aqueous extract of PHF
Tannins	+++	++	++
Flavonoids	++++	++++	+++
Steroids	++	+	-
Alkaloids	++	+	+
Saponins	+	+	++
Total phenols	++++	+++	+
Carbohydrates	+++	+++	+++
Glycosides	+++	++	-

++++ Appreciable amount (positive within 3 min); +++ Appreciable amount (positive within 5 min); ++ Moderate amount (positive after 5 min within 10 min); + Trace amount (positive after 10 min within 15 min); - non traceable amount.

DPPH RADICAL SCAVENGING CAPACITY

DPPH free radicals generally used for investigation of preliminary radical scavenging activity of the plant extract.[20] Scavenging of DPPH radical is correlated to the inhibition of lipid peroxidation.[21] DPPH used as a substance to estimate the antioxidant activity.[22] Antioxidants either transfer an electron or a hydrogen atom to DPPH thus counteracting its free radical appeal.[23] DPPH test, a stable free radical, to decolorize in the occurrence of antioxidants, is a direct and reliable method for determining radical scavenging action.[24]

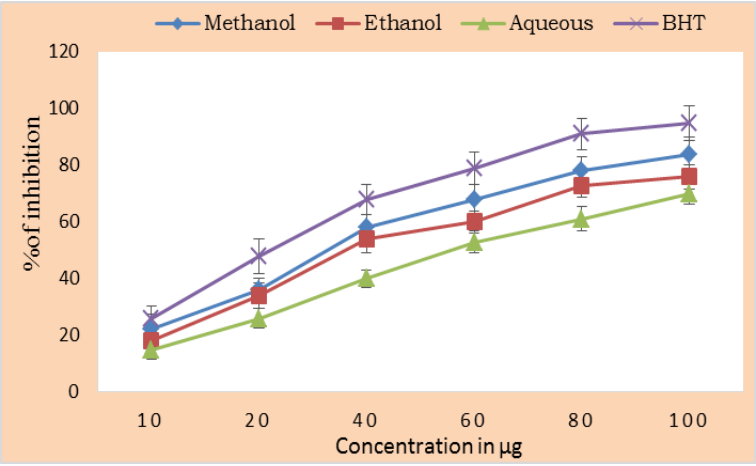

Fig. 1: DPPH radical-scavenging activities of methanolic, ethanolic and aqueous extracts of Polyherbal formulation and standard (BHT). Values are means ± SD of three determinations.

Figure. 1 illustrates a significant reduction in the concentration of DPPH radical due to the scavenging ability of the extracts. Butylated hydroxyltoluene (BHT) were used as positive controls presented the highest activity at all concentrations. The IC_{50} values (the concentration with 50% scavenging activity) of scavenging activities on DPPH radical were found to be 32.87 µg mL^{-1}, 50.19 µg mL^{-1}, 71.21 µg mL^{-1} and 18.87 µg mL^{-1} for methanolic extract, ethanolic extract, aqueous extract and BHT respectively (**Table 2**). The DPPH radical scavenging activity was found to be in the order of BHT > methanolic extract > ethanolic extract >aqueous extract. The DPPH assay basically used as a quick, reliable and reproducible parameter to search the *in vitro* general antioxidant activity of pure compounds as well as plant extracts.[25] From the results it is known that the polyherbal formulation possess hydrogen donating abilities for methanolic, ethanolic and aqueous extract and does scavenging free radicals.

Table 2: IC_{50} radical scavenging activity of methanolic, ethanolic and aqueous extract of Polyherbal formulation and control (BHT). Values are means ± SD of three determinations.

Compounds	DPPH radical scavenging activity	Nitric oxide radical scavenging activity	Superoxide anion radical scavenging activity	Hydroxyl radical scavenging activity	Lipid peroxidation inhibition activity
Methanol	32.87 ± 0.31	47.66 + 0.28	62.78 ± 0.53	60.12 ± 0.39	57.89 ±0.41
Ethanol	50.19 ± 0.45	58.67 ± 0.38	65.74 ± 0.44	68.45 ± 0.44	65.17 ±0.48
Aqueous	71.21 ± 0.68	73.98 ± 0.49	77.16 ± 0.61	75.61 ±0.51	79.76 ± 0.52
BHT	18.87 ± 0.13	19.26 ± 0.12	18.09 ± 0.18	36.26 ± 0.25	24.55 ± 0.17

NITRIC OXIDE RADICAL SCAVENGING CAPACITY

Nitric oxide is a vital biochemical mediator produced by endothelial cells, macrophages, neurons, etc. and is involved in the directive of various physiological processes, excess concentration of nitric oxide is related with several diseases.[26] Nitric oxide and superoxide anion causes damage and toxicity as they produce reactive peroxynitrite, which leads to severe toxic reactions with biomolecules like protein, lipids and nucleic acids.[27] **Figure. 2** shows the nitric oxide scavenging activities of the polyherbal formulation and its plants constituents with IC_{50} values in (**Table 2**). The inhibition reactions were especially rapid at lower concentrations (<10 µg/ml), slowing down considerably at higher concentrations (100 µg/ml). Compared to aqueous extract (61.67%) the best NO scavenging activity was obtained from methanolic extract (80.83%) at the concentration of 100 µg/ml. The plant ingredients have the properties to work against the effect of nitric oxide formation and in turn may be of significant interest in relation to preventing the ill effects of extreme NO generation in the human body. Our findings suggest that polyherbal formulation have the property to counteract the effect of NO formation due to the presence of tannins and f1avonoids and in turn may be of substantial interest in preventing the ill effects of excessive NO generation.

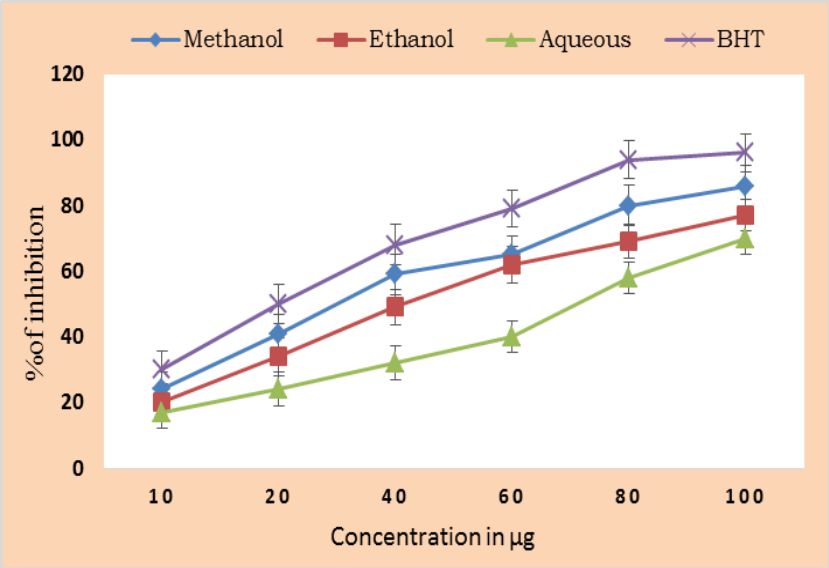

Fig. 2: Nitric oxide radical-scavenging activities of methanolic, ethanolic and aqueous extracts of Polyherbal formulation and standard (BHT). Values are means ± SD of three determinations.

SUPEROXIDE RADICAL SCAVENGING CAPACITY

Superoxide anion a harmful reactive oxygen species as it damages cellular components in biological systems. A number of enzyme systems produce this species in auto-oxidation reactions and by non-enzymatic electron transfers that diminish molecular oxygen. It can also reduce certain iron complexes such as cytochromes.[28] **Figure. 3** shows the superoxide anion radical scavenging activity of polyherbal formulation in methanolic, ethanolic, aqueous and BHT. Significant superoxide anion radical scavenging activities were appeared at all the tested concentrations of the PHF. The scavenging activity increased with increasing concentration, the order of the superoxide anion radical-scavenging activity was BHT > methanolic extract > ethanolic extract > aqueous extract. The IC_{50}, as shown in (**Table 2**), was found to be 62.78, 65.74, 77.16 and 18.09 mg mL^{-1} for methanolic extract, ethanolic extract, aqueous extract and BHT respectively. The results suggest that the compound exhibit scavenging effect on superoxide anion radical generation that could help to prevent oxidative damage. Levels of O_2 may rise substantially when the cells are exposed to oxidative stress, causing oxidative damage to

DNA, oxidation of proteins and lipid peroxidation.[28] Hence this assay tells us a grave physiological way to measure the antioxidant activity of the plant extracts and to compare with standard compound.[29]

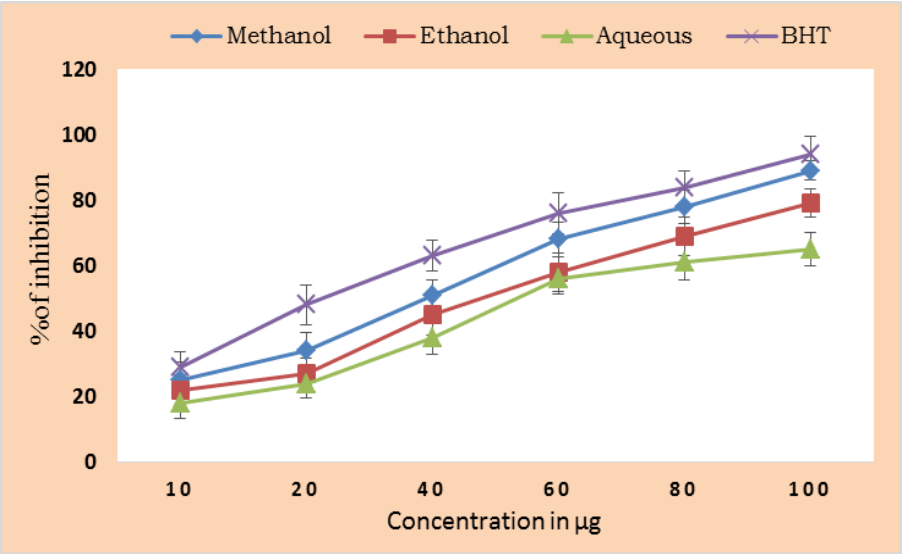

Fig. 3: Superoxide radical-scavenging activities of methanolic, ethanolic and aqueous extracts of Polyherbal formulation and standard (BHT). Values are means ± SD of three determinations.

HYDROXYL RADICAL SCAVENGING CAPACITY

Hydroxyl radical is a desperate reactive species formed in biological systems. It is capable of injurious almost every molecule found in living cells. This radical has the ability to join nucleotides in DNA and cause strand breakage, which contributes to carcinogenesis, mutagenesis and cytotoxicity. In addition, hydroxyl radical is considered to be one of the quick initiators of the lipid peroxidation process, abstracting hydrogen atoms from unsaturated fatty acids.[30] **Figure. 4**, all the samples exhibited effective or moderate activity in an amount dependent manner. The highest scavenging activity was found in methanolic extract, it showed 71.33% scavenging activity at the concentration of 100 µg/ml, while that of aqueous extract was determined to be 59.50% at the same concentration. The IC_{50} values were found to be 60.12, 68.45, 75.61 and 36.26 mg mL^{-1} for methanolic extract, ethanolic extract, aqueous extract and BHT respectively (**Table 2**). Hence, the pure antioxidant with the lowest number of hydroxyl groups displayed the highest activity towards hydroxyl radicals.

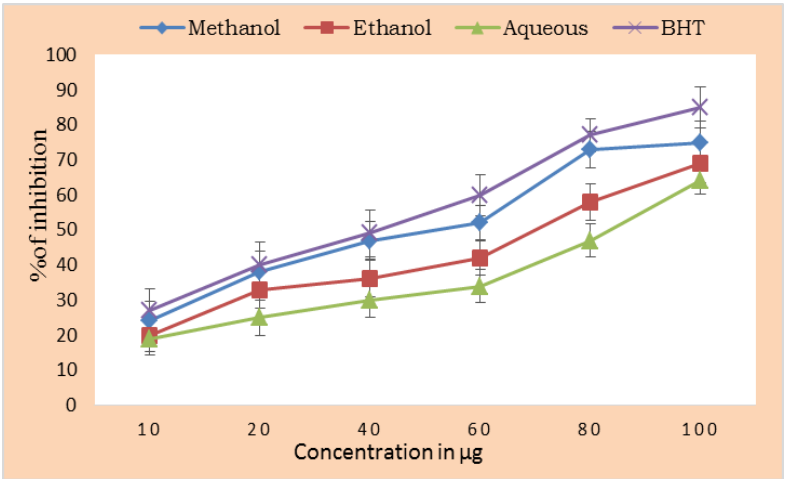

Fig.4: Hydroxyl radical-scavenging activities of methanolic, ethanolic and aqueous extracts of Polyherbal formulation and standard (BHT). Values are means ± SD of three determinations.

LPO RADICAL SCAVENGING CAPACITY

Oxygen radicals initiated the peroxidation of membrane lipids, which may lead to cell injury. Initiation of lipid peroxidation (LPO) by ferrous sulfate takes place moreover through a ferryleperferryl complex.[31] or through an OH radical by the Fenton reaction, thereby initiating a tumble of oxidative reactions.[32] The efficacy of LPO scavenging activity of methanolic, ethanolic and aqueous extracts and controls were shown in **figure. 5**. In the present investigation, the methanolic extract

registered the highest LPO scavenging activity (78.17%) while the aqueous extract showed a lower level LPO scavenging activity (59.50%). This may be due to the antioxidants in the PHF offer resistance to oxidative stress by several mechanisms, including scavenging free radicals and inhibiting lipid peroxidation, thereby preventing disease.[33] The IC_{50} values were found to be 57.89, 65.17, 79.76 and 24.55 mg mL^{-1} for methanolic extract, ethanolic extract, aqueous extract and BHT respectively (**Table 2**).

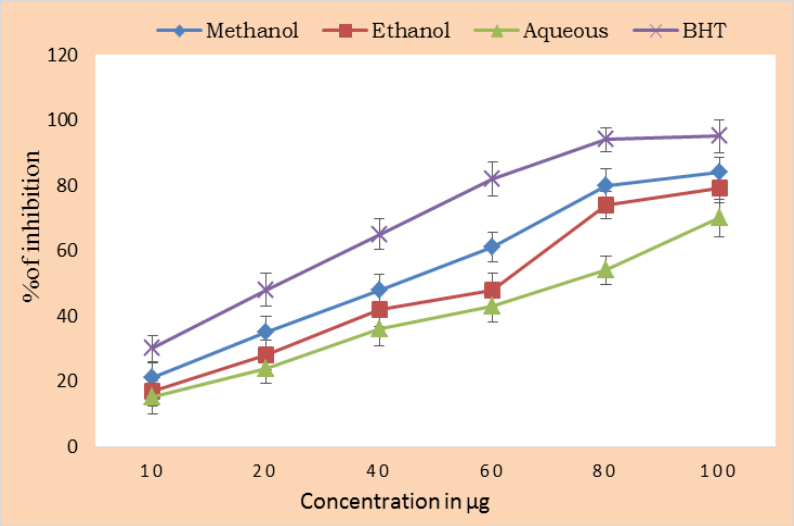

Fig. 5: Lipid peroxidation scavenging activities of methanolic, ethanolic and aqueous extracts of Polyherbal formulation and standard (BHT). Values are means ± SD of three determinations.

TOTAL PHENOLICS CONTENT

Phenolic compounds are known as powerful chain breaking antioxidant.[34] because of their scavenging ability they are very important plant constituents, which is due to their hydroxyl groups.[35] In methanolic, ethanolic and aqueous extracts of Polyherbal formulation the total phenolic content was found to be (23.56 ± 1.16 mg of gallic acid), (19.26 ± 0.84 mg of gallic acid) and (12.45 ± 0.71 mg of gallic acid) respectively in terms of gallic acid equivalent (**Table 3**). In addition it has been determined that the highest extraction yield was found in methanolic extract compared to ethanol and aqueous.

TOTAL FLAVONOIDS CONTENT

Flavonoids are a collection of polyphenolic compounds, which display several biological effects such as anti-inflammatory, anti-hepatotoxic, anti-ulcer, anti-allergic, anti-viral and anti-cancer activities.[36] Because of their phenolic hydroxyl groups, flavonoids are capable of effectively scavenging the reactive O2 species and so they potent antioxidants activity.[37] The total flavonoids content of methanolic, ethanolic and aqueous extracts of Polyherbal formulation was determined to be (21.84 ± 1.03 mg of rutin), (18.86 ± 0.92 mg of rutin) and (13.22 ± 0.82 mg of rutin) respectively in terms of quercetin equivalent (**Table 3**).

Table 3: Total phenols and total flavonoids of methanolic, ethanolic and aqueous extract of Polyherbal formulation. Values are means ± SD of three determinations.

Compounds	Total phenol (equivalent to mg of gallic acid)	Total flavonoids (equivalent to mg of rutin)
Methanol	23.56 ± 1.16	21.84 ± 1.03
Ethanol	19.26 ± 0.84	18.86 ± 0.92
Aqueous	12.45 ± 0.71	13.22 ± 0. 82

TOTAL ANTIOXIDANT ACTIVITY

The phospho molybdenum method is based on the reduction of Mo(VI) to Mo(V) by the antioxidant composition and the formation of a green phosphate/Mo(V) complex with a maximal absorption at 695 nm. Increase in absorbance indicated the increase amount of total antioxidant capacity. The antioxidant capacities of methanolic extract, ethanolic extract and aqueous extracts were measured spectrophotometrically. Potent antioxidant capacity has been found in all samples. The results shown in **Figure 6**. The methanolic extract exhibited the highest activity of all (49.28 ± 3.33 mg α -tocopherol per g), than ethanolic extract (42.77 ± 3.84 mg α-tocopherol per g) and aqueous extract (24.36 ± 2.95 mg α -tocopherol per g).

REDUCING POWER PROPERTY

It is believed that antioxidant activity and reducing power are related.[38] Reductones inhibit lipid peroxidation by donating a hydrogen atom and thereby terminating the free radical chain reaction.[39] Reducing power assay is a convenient and rapid screening method

for measuring the antioxidant potential.[40] The reduction ability ("Fe^{3+} to Fe^{2+} transformation" in terms of increasing absorbance) was found to increase with rising concentration in all the samples. The color of the test solution then changes from yellow to different shades of green and blue.[41] The ability to reduce Fe (III) may be attributed to the hydrogen donating effect of phenolic compounds.[42] In the present study, 33.44 ± 2.88 mg, 29.84 ± 2.32 and 18.85 ± 2.12 mg of the methanolic extract, ethanolic extract and aqueous extract showed antioxidant activity as is depicted in **Figure 6**.

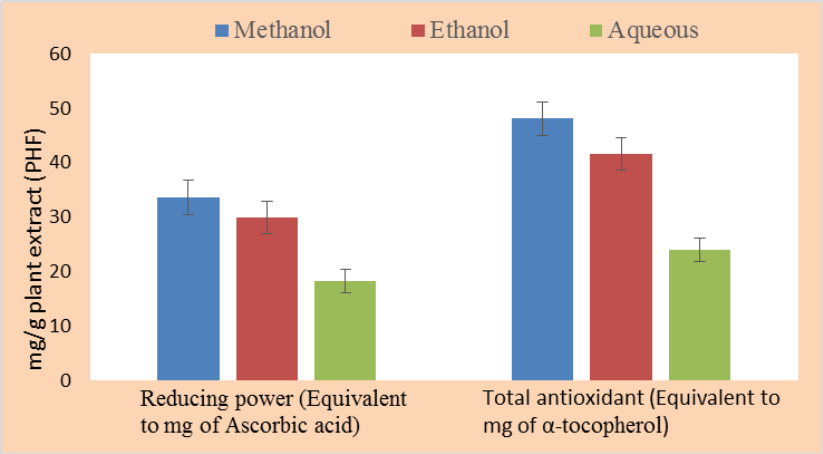

Figure 6: Total antioxidant activity and reducing power of methanolic, ethanolic and aqueous extract of Polyherbal formulation. Values are means ± SD of three determinations.

CONCLUSION

From the above results, it is concluded that the methanolic extract of the polyherbal formulation displayed more potent *in vitro* antioxidant activity, with higher percentage of inhibition than the ethanolic and aqueous extract of the polyherbal formulation. Phytochemical analysis of polyherbal formulation showed the presence of alkaloids, phenol, flavonoid, glycosides, saponins, steriods and tannin compounds in the extracts with higher amount of flavonoid and phenol. These phytochemical constituents might be responsible for radical scavenging activity. The above data indicates that polyherbal formulation extracts could be great significance for the treatment of radical related diseases. Additional studies are in evolution to isolate the active principles from the extracts and to reveal the particular mechanism of action for free radical scavenging effect.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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