MSW MANAGEMENT -Multidisciplinary, Scientific Work and Management Journal
ISSN: 1053-7899
Vol. 36 Issue 1s, 2026, Pages: 3681-3683

Investigation of Anti-Oxidant and Anti-Hyperlipidemic Activity of Some Plants

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Abstract-

We evaluated the anti-Hyperlipidemic and Anti-Oxidant activity of Some Herbal Plants. We tested the methanol extract of *Cymbopogon flexuosus, Zingiber officinale,* and *Terminalia arjuna* anti-Hyperlipidemic activity against Dexamethasone induced hyperlipidemia in Wistar rodents, *Cymbopogon flexuosus, Zingiber officinale,* and *Terminalia arjuna* has excellent anti-Hyperlipidemic action. We further measured the *Cymbopogon flexuosus, Zingiber officinale,* and *Terminalia arjuna* DPPH assay and Hydrogen Peroxide radical scavenging effect for in vitro analysis; Methanolic extract shown the potent anti-oxidant (DPPH; IC_{50}= 25 µg/ml, HPSA; IC_{50}= 54 µg/ml) suggesting its potential as a natural antioxidant. Because of its phytochemical content, *Cymbopogon flexuosus, Zingiber officinale,* and *Terminalia arjuna* may have anti-Hyperlipidemic and antioxidant activities These plants were found to be potent enough to be studied further for its in vivo anti-Hyperlipidemic and anti-oxidant to perform formulations.

Keywords- Dexamethasone, Antioxidant, Herbal Plants, Methanol extract.

Introduction

Hyperlipidemia is described by raised serum levels of total cholesterol (TC), low- density lipoprotein (LDL), Very low -density lipoprotein (VLDL), and diminished serum level of high density- lipoprotein (HDL). According to American heart incorporation, a high level of fats known as hyperlipidemia. These fats consist of cholesterol and triglyceride. Lipids and fatty substances in the blood and is a greater risk factor in the growth of atherosclerosis and heart diseases[1]. Instead of gallstones, pancreatitis, or xanthomas, hyperlipidemia poses the same risk as CAD, MI, or hypertension[2]. Hyperlipidaemia CAD has the potential to overtake all other causes of mortality in India[3]. As a result of an overreaction to several types of harmful stimuli to the arterial wall, hyperlipidemia develops as a primary complication of coronary artery disease[4]. Rigveda, Yajurveda, Atharvaveda, Charak Samhita, and Sushrut Samhita are some of the classic Indian medical texts that detail the medicinal uses of plants components. We looked at the chemical properties and potential health benefits of the decorative plants *Cymbopogon flexuosus, Zingiber officinale,* and *Terminalia arjuna* (using a methanol extract). In particular, we determined the phytochemical composition of the plant and tested its methanol extract for antihyperlipidemic and antioxidant characteristics. These plants' methanol extract includes wide variety of phytochemicals that may have medicinal uses. The extract also showed promising results against antihyperlipidemic and antioxidant *activities*[5].

Materials and techniques

Verification of Plant Collection and Extract Preparation: The plant *Cymbopogon flexuosus, Zingiber officinale,* and *Terminalia arjuna* leaves and bark were obtained from Maharana Pratap College of Pharmacy's herbal Garden and validated by botanist.

Preparation of Methanolic extract: The plants component was cleaned and then left to dry for a week after garbage removal. In a grinder, the dried herb was roughly ground. A sealed container was used to store the powdered sample in a cold, dark, and dry location prior to testing. A clean glass container with a flat bottom was used to steep 350 g of powder in 1500 mL of methanol. Stir the contents of the sealed jar every day for ten days. The mixture was roughly filtered using a cleaned white cotton towel. The process was carried out using Whatman filter paper. Use a revolving vacuum evaporator to dry and concentrate the filtrate. As a result, 33 g of gummy concentrate (13.43% yield) and crude methanol were generated[6-7].

Chemicals and drugs: The all-analytical grade ingredients were acquired from Merck Darmstadt, Germany etc.

In Vitro Anti-oxidant: The extracts were tested for their antioxidant properties in vitro using the following techniques.

DPPH Assay: 1, 2-Diphenyl-2-picryl hydrazyl Radical (DPPH)

0.4 mL of 0.3M DPPH reagent made in ethanol was combined with 0.1 mL of samples at different concentrations. After giving the mixture a good shake, it was allowed to sit at room temperature for half an hour in the dark. Spectrophotometric measurements of the reaction's absorbance at 517 nm were made both immediately after mixing and later during incubation. The following formula was used to determine the DPPH free radical's scavenging effect[8].

Abs (control) − Abs (standard) / Abs (control) × 100 = % Scavenging activity

where sample absorbance is the absorbance of the test extract and control is the absorbance of the blank (a reaction with all the reagents except the test extract). To determine 50% inhibition (IC_{50}), tests were run in triplicate. utilising toluene that has been butylated.

Assay for Hydrogen Peroxide Scavenging (HPSA)

The Ruch technique was used to assess the extracts' capacity to scavenge hydrogen peroxide. Hydrogen peroxide (2 mmol/l µg/lt) was made into a solution in phosphate buffer (PH 7.4). Hydrogen peroxide solution (0.6 ml) was mixed with extracts at different concentrations (10–100 µg/ml). After 10 minutes, the absorbance at 230 nm was measured in comparison to a blank solution that contained phosphate buffer but no hydrogen peroxide[9].

% Scavenging activity =Abs (control) - Abs (standard) / Abs (control) × 100

Experimental Rodents

For this investigation, 150-200g Wistar albino rats of either sex was employed. The Institutional Animal Ethics Committee bought them. Polypropylene pens housed animals (3 per pen) at 28 ±50°C and a 12 h light/dull cycle. Hindustan The animal was fed liver chow pellets, and the water was not basic. Animals were fasted for a medium period before the exam, and the study was authorized by IAEC across all research frameworks.

In vivo anti-hyperlipidemic activity

Dexamethasone Induced Hyperlipidemia in Rodents

Dexamethasone, a glucocorticoid, is known to elevate plasma lipid levels, resulting in hyperlipidemia. Dexamethasone (10 mg/kg/day, subcutaneously) was provided to Wistar rats for 8 days to affect hyperlipidemia. The creatures were categorized into five groups, with each group including six Wistar rodents (n=6).

- ✓ Group 1 (Normal control) - Administered normal saline solution
- ✓ Group 2 (Hyperlipidemic control) - Administered normal saline solution
- ✓ Group 3 (Standard group) – Gemfibrozil 10 mg/kg/ day I.P. suspended in gum acacia in water
- ✓ Group 4 (Test group-I) – Methanol Extract of *Cymbopogon flexuosus, Zingiber officinale,* and *Terminalia arjuna* 30 mg/ kg orally
- ✓ Group 5 (Test group- II) - Methanol Extract of *Cymbopogon flexuosus, Zingiber officinale,* and *Terminalia arjuna* 60 mg/kg orally

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To induce hyperlipidemia, all of the rats in groups II–V were given a subcutaneous injection of Dexamethasone (10 mg/kg/day S.C.) for a duration of 8 days. Gemfibrozil (10 mg/kg/day) was administered to Group III rats, whereas normal saline was given to the animals in the normal hyperlipidemia control groups. The oral method was used to administer methanol extracts of *Cymbopogon flexuosus, Zingiber officinale,* and *Terminalia arjuna* to rats in Groups IV and V, respectively, at dosages of 30 mg/kg/day and 60 mg/kg/day for the duration of the 8-day trials. After the trial was finished, the rats that had gone without food overnight were beheaded while under light ether anesthesia so that blood could be obtained. Extracted serum and studied lipid profiles, which are biochemical parameters[10].

Biochemical Analysis: Lipids in plasma Total cholesterol TG HDL cholesterol Very low-density lipoprotein Serum samples were tested for low-density lipoprotein (LDL) using Qualigens diagnostic Mumbai kits. The samples were semiautomatically examined.

Statistical Analysis: Statistics were done with GraphPad 5.0. Data were recorded as mean ± S.E.M. Dunnett's test and analysis of variance (ANOVA) determined group variation's statistical significance.

Results and Discussion:

Activities of antioxidants: Numerous studies have demonstrated the role of oxidative stress in age-related neurodegenerative disorders. The beneficial effects of antioxidants to prevent or lessen neuronal death that occurs in the pathophysiology of this condition have also been the subject of several research studies. Furthermore, a compound's overall antioxidant activity and capacity to scavenge radicals are indicators of its antioxidant potential. Two activities were assessed to ascertain the plant extracts' capacity to function as antioxidants: the Hydrogen Peroxide Scavenging assay and their capacity to scavenge DPPH.

DPPH radical scavenging activity: The anti-oxidant activity of many extracts was investigated utilising the 1, 2-diphenyl-2 picryl hydrazyl radical (DPPH). Table 1 discusses the findings.

Table 1: DPPH Radical Scavenging activity of various extract

S.No	Concentration (µg/ml)	% Inhibition	
		Standard (Butylated hydroxyl Toluene)	Methanol Extract of *Cymbopogon flexuosus, Zingiber officinale,* and *Terminalia arjuna*
1.	20	53.95	45.94
2.	40	64.81	59.86
3.	60	76.62	68.18
4.	80	85.21	76.95
5	100	91.45	88.87
IC_{50} (µg/ml)		10	25

The DPPH is a popular tool for testing the free radical scavenging action since it is a stable free radical that can receive a proton from any molecule and generate a rich purple hue. A change in hue causes DPPH's absorbance at 517 nm to drop as its concentration drops. The IC_{50} value (µg/ml) of 25 values of standard BHT was 10 in the methanol extract of *Cymbopogon flexuosus, Zingiber officinale,* and *Terminalia arjuna. Cymbopogon flexuosus, Zingiber officinale,* and *Terminalia arjuna*were discovered to have high scavenging action when compared to standard, when extracted in methanol.

Radical Scavenging by Hydrogen Peroxide: The Methanolic extract and *Cymbopogon flexuosus, Zingiber officinale,* and *Terminalia arjuna* extracts were subjected to hydrogen peroxide radical scavenging assay and the results are tabulated below in Table 2.

Table 2: Hydrogen peroxide Radical Scavenging activity of various extract

S. No	Concentration	% inhibition	
		Standard (Ascorbic acid)	Methanol Extract of *Cymbopogon flexuosus, Zingiber officinale,* and *Terminalia arjuna*
1	20 µg/ml	32.23	40.43
2	40 µg/ml	38.13	45.65
3	60 µg/ml	56.34	50.81
4	80 µg/ml	73.32	57.65
5	100 µg/ml	88.34	65.56
IC_{50} (µg/ml)		47	54

Using this procedure, we could find out how well the extract scavenged hydrogen peroxide. The Methanolic extract had an IC_{50} value of 54 when the absorbance was measured at 230 nm and the concentration in microgrammes per millilitre was computed. The normal ascorbic acid IC_{50} value was 47.

Results of Total Cholesterol and Total TGs. The hyperlipidemia-induced group has much higher total cholesterol than normal rats. Values rose to 117.71 ± 1.329 mg/dl compared to Group I (normal rat group) at 64.43± 0.933 mg/dl. Hypercholesteremia. Treatment with Methanolic extract *and Cymbopogon flexuosus, Zingiber officinale,* and *Terminalia arjuna* (30 mg/kg) or v (60mg/kg) reduces values to 84.23 ±1.046 (P< 0.001) and 82.35 ± 0.885mg/dl (P<0.0001). The Methanolic extract and *Cymbopogon flexuosus, Zingiber officinale,* and *Terminalia arjuna* treatment group significantly lower total cholesterol. In addition, Gemfibrozil significantly reduced blood total cholesterol levels to 73.70 ± 0.794 mg/dl (P< 0.001) [Table-3]. In the dexamethasone-induced group, TG levels reached 150.71 ± 0.518 mg/dl, compared to 63.75 ± 0.507 mg/dl in normal rats. Triglyceridemea. Methanolic extract and *Cymbopogon flexuosus, Zingiber officinale,* and *Terminalia arjuna* 30 mg/kg and mg/kg significantly reduced values to 79.50 ± 0.526 mg/dl (P<0.001) and 75.25 ± 0.641 mg/dl (P<0.0001). In the Gembibrozil-treated group (Std. Group), levels dropped to 68.33 ± 0.572 mg/dl (P<0.001) [Table-3].

Results of High-density Lipoprotein (HDL) Cholesterol. A dexamethasone-induced group had significantly lower HDL cholesterol than normal rats. The results dropped to 24.75±0.410 mg/dl compared to 40.68 ±0.711 mg/dl in the normal rat group. In the group prevented with Methanolic extract *Cymbopogon flexuosus, Zingiber officinale,* and *Terminalia arjuna* 30 mg/kg and 60 mg/kg, The results were 25.79 ± 0.602 (P<0.001) and 28.40 ± 0.517 mg/dl (P<0.0001). In Gemfibrozil-treated group (Std.Group), values were 34.50±0.665 mg/dl (P<0.001) [Table-3].

Results of LDL-cholesterol and VLDL- Cholesterol. The dexamethasone-induced group had significantly higher LDL-cholesterol levels (56.32 ± 0.811 mg/dl) compared to the normal rat group 14.59 ± 0.495. In the group prevented with Methanolic extract and *Cymbopogon flexuosus, Zingiber officinale,* and *Terminalia arjuna* 30 mg/kg and 60 mg/kg. The readings decreased by 32.67 ± 0.609 (P<0.001) and 26.73 ± 0.551mg/dl (P<0.0001). LDL cholesterol drops significantly under extract therapy. Gemfibrozil significantly reduced LDL-cholesterol to 23.35 ± 0.563 mg/dl (P<0.001). [Table-3]. The dexamethasone-induced group had significantly higher VLDL-cholesterol levels (38.42 ± 0.650 mg/dl) compared to the normal rat group (13.42 ± 0.455) in the group prevented with Methanolic extract and *Cymbopogon flexuosus, Zingiber officinale,* and *Terminalia arjuna* 30 mg/kg and 60 mg/kg. The results decreased by 30.60 ±0.441 (P<0.001) and 25.80 ± 0.505 mg/dl (P<0.0001). Extract treatment group is much lower. Gemfibrozil significantly reduced VLDL-cholesterol to 19.75 ± 0.527 mg/dl (P<0.001). [Table-3].

Results of Atherogenic Index. In the normal rodent group, the atherogenic index is 1.58, but in the dexamethasone-induced group, it increases to 4.89. Important reductions to 3.26 and 2.89 were observed in the groups that were pretreated with Methanolic extract and *Cymbopogon flexuosus, Zingiber officinale,* and *Terminalia arjuna* 30 mg/kg and 60 mg/kg, respectively. The readings of 2.13 have been significantly reduced with gemfibrozil [Table-3].

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Table 3: Anti-hyperlipidemic Effect of Methanolic extract and *Cymbopogon flexuosus, Zingiber officinale,* and *Terminalia arjuna* in Wistar Rodents

Group	Treatment/dose	Total cholesterol (mg/dl)	Total TG (mg/dl)	HDL-Cholesterol (mg/dl)	LDL-Cholesterol (mg/dl)	VLDL-Cholesterol (mg/dl)	Atherogenic index
I	Normal-group	64.43 ± 0.933	63.75 ± 0.711	40.68 ± 0.795	14.59± 0.495	13.42 ± 0.455	1.58
II	Normal- control group	117.71 ± 1.329	150.71 ± 0.518	24.75 ± 0.410	56.32 ± 0.811	38.42 ±0.650	4.89
III	Standard group Gemfibrozil (10mg/kg)	73.70 ± 0.794**	68.33 ± 0.572**	34.50 ± 0.665**	23.35 ± 0.563**	19.75 ± 0.527**	2.13
IV	Methanolic extract and *Cymbopogon flexuosus, Zingiber officinale,* and *Terminalia arjuna* (30mg/kg) -I	84.23 ± 1.046 *	79.50 ± 0.526**	25.79 ± 0.602**	33.6 7± 0.609**	30.60 ± 0.441**	3.26
V	Methanolic extract and *Cymbopogon flexuosus, Zingiber officinale,* and *Terminalia arjuna* (60mg/kg)-II	82.35 ± 0.885***	75.25 ± 0.641***	28.40 ± 0.517***	26.73 ± 0.55***	25.80 ± 0.505***	2.89

All results were presented as mean± SEM. All data were evaluated using one-way ANOVA and Dunnett's multiple comparison test, with significance determined at P<0.05. *** P<0.0001 vs. control group.

DISCUSSION

Atherosclerosis, which in turn causes coronary heart disease (CHD), is a direct outcome of hyperlipidemia, which is a major global health risk factor. 60% of all cardiovascular cases in 2020 will come from India, according to the World Health Organization. Hyperlipidemia affects and damages several bodily systems. This, in turn, alters the functioning of cells, resulting in cell death and various diseases. Obesity may develop as a result of consuming a high-fat diet that raises cholesterol levels. Elevated levels of bad cholesterol (LDL), particularly very low-density lipoprotein (VLDL), increase the danger of cardiovascular disease (CHD). Lowering HDL cholesterol levels reduces coronary heart disease risk. Decreases in CHD risk of 2-3% are associated with reductions of 1% in cholesterol. The metabolic syndrome (syndrome-x) increases the risk of cardiovascular mortality and morbidity in patients with hyperlipidemia, hypertension, and diabetes mellitus. The etiology of coronary heart disease, which is caused by increasing atherosclerosis, is influenced by several factors, including but not limited to diabetes, hypertension, smoking, glucocorticoids, nutritional factors, and psychological factors. Coronary heart disease is the leading cause of death and disability worldwide. Over eighty percent of obese people may not be able to successfully manage their weight with high-level motivational direction that incorporates innovations in food and lifestyle. This finding is supported by another research. Thence, particular medicines are needful. Despite almost research exertion, only not many such medicines are beneficial, and they are companioned by adverse drug effects. The United States Food and Drug Administration (FDI) has recommended some anti-obesity medicines, but these medicines have caused high incidences of cardiovascular and depressive actions. Few such medicines have been removed from the pharmaceutical market. Because of these side effects, natural products are existence investigated as safer choices. Crude herbal extracts and isolated phytoconstituents can stimulate bodyweight reduction and obstruct diet influenced obesity. DPPH assay and Hydrogen Peroxide radical scavenging effect for in vitro analysis; Methanolic extract shown the potent anti-oxidant (DPPH; IC50= 25 µg/ml, HPSA; IC50= 54 µg/ml) suggesting its potential as a natural antioxidant. Because of its phytochemical content, *Cymbopogon flexuosus, Zingiber officinale,* and *Terminalia arjuna* may have hyperlipidemic activity and antioxidant activities. Methanolic extract and *Cymbopogon flexuosus, Zingiber officinale,* and *Terminalia arjuna* show anti-atherogenic action through inhibition of NF-KB activation and the resulting formation of inflammatory cytokines (TNF-α and IL-6) reactive oxygen species, and another inflammatory agent in a PDE-1-independent manner. Athyros *et al,* 2011 from the above information, we decided to explore the anti-hyperlipidemic activity and in vitro antioxidant activity of Methanolic extract and *Cymbopogon flexuosus, Zingiber officinale,* and *Terminalia arjuna* in Wistar rodents as we also wanted to know the possible implication of Methanolic extract and *Cymbopogon flexuosus, Zingiber officinale,* and *Terminalia arjuna* drug in the management of hyperlipidemia.

CONCLUSIONS

The findings indicate that the all-plant extracts have immediate Anti-hyperlipidemic and antioxidant properties, supporting the traditional usage of this plant portion to treat Anti-hyperlipidemic and antioxidant

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