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EVALUATION OF ANTI-HYPERLIPIDEMIC POTENTIAL OF SYZYGIUM CUMINI & HELIANTHUS ANNUUS SEEDS IN THE SUITABLE EXPERIMENTAL ANIMAL MODEL

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ABSTRACT

This research highlights the potential of medicinal herbs like Helianthus annus and Syzygium cumini to reduce cholesterol levels in the body, which might be important in preventing cardiovascular issues such as atherosclerosis. Significantly, when these herbs were administered at a dose of 0.75 + 0.75 ml/kg, there was a significant decrease in cholesterol levels. Nevertheless, more inquiries are necessary to thoroughly examine the whole range of advantages obtained from these oils extracted from seeds. Polyphenols, which are recognized for their ability to prevent oxidation, may possibly hinder the oxidation of LDL cholesterol, thus leading to the observed reduction in lipid levels. Despite the lack of a clear understanding of the exact mechanism, it is important to further investigate the beneficial effects of polyphenols. This highlights the need for more scientific investigation to clarify the underlying processes. The blend of these botanical extracts produced encouraging hypolipidemic outcomes, as shown by its impact on cholesterol levels in a Triton-induced rat model. Certain results provide a fundamental comprehension of the possible curative advantages of certain botanical extracts, setting the basis for future investigations in this field.

KEYWORDS: This highlights the need for more scientific investigation to clarify the underlying processes.

INTRODUCTION

Hyperlipidemia is characterised by elevated levels of total cholesterol (TC), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), triglycerides, and decreased levels of high-density lipoprotein (HDL) in the bloodstream. "Hyperlipidemia is a significant risk factor that contributes to the high occurrence and seriousness of atherosclerosis and the resulting coronary heart disease." (Ginghina et al., 2011). The liver is responsible for the synthesis of about two-thirds of the total cholesterol produced in the body (Jorgensen et al., 2013, Ginghina et al., 2011). The enzyme that limits the pace of a biochemical reaction is known as the rate-limiting enzyme. In the case of cholesterol synthesis, the rate-limiting enzyme is 3-hydroxy-3-methyl glutaryl (HMG)-Co A reductase. This enzyme plays a crucial role in regulating the quantity of cholesterol in cells via feedback mechanisms. The management of hyperlipidemia include the implementation of dietary restrictions, regular physical activity, as well as the administration of lipid-lowering diets and medications. The primary medications used to treat hyperlipidemia are hydroxymethylglutarate Coenzyme A (HMG-CoA) reductase inhibitors, popularly known as statins (Jorgensen et al., 2013). Other medicines used to treat hyperlipidemia include bile acid sequestrants such as colestipol, fibrates such as clofibrate and fenofibrate, and cholesterol absorption inhibitors such as ezetimibe and omega-3-fatty acids. Although there are several medications available for treating hyperlipidemia, antihyperlipidemic therapy currently lacks effectiveness and safety. Statins, which are beneficial in reducing LDL, have a potential danger of causing significant muscle damage. "Niacin, an effective medication for reducing triglyceride levels, has the potential to induce hyperglycemia and liver damage" (Mishra et al., 2011). The administration of fibrates often results in adverse consequences that often affect the liver, kidneys, or skeletal muscle. Hence, there is still a need for the development of efficient antihyperlipidemic medications (Jeyabalan et al., 2009). Plant-based goods are often regarded as less harmful and devoid of adverse effects when compared to synthetic products (Brouwers et al., 2012).

MATERIALS AND METHODOLOGY

Collection and Authentication of Plant Material

The seeds of Syzygium cumini (L) Skeels and H.annus were acquired from a herbal shop in Mawana, Meerut, India.

The raw botanical material was verified by NISCAIR, New Delhi, using a voucher specimen number:

Preparation of Seed Oil using Cold press

The seeds *Syzygium cumini (L) Skeels* and *H.annus were* sun dried and grinded into a coarse powder. In oil extraction the seeds of each plant was The seeds were subjected to mechanical pressure at a stable room temperature of 25°C without any thermal processing. After compression, the seeds were left undisturbed for a period of 24 hours at the same temperatureto facilitate oil extraction. The separated oil was then meticulously purified filtering using Whatman No.4 filter paper and a glass funnel.

Phytochemical Investigation

The *Syzygium cumini (L) Skeels* and *H.annus* The oil was analyzed through phytochemical testing to identify various plant-based compounds, including carbohydrates, alkaloids, glycosides, steroids, tannins, and others.

Recognisance of Carbo-hydrates

100mg of extract was dissolved in 5ml of H2O & then solution was filtered. The resulting filtrate was put to following tests:

Molish's Test - 2-3 droplets of a 2% w/v solution of -naphthol were added to 2ml of filtrate. This mixture was aggressively agitated before 1ml of conc. H2SO4 was introduced slowly along the sides of test container & allowed to rest. At interface b/w two layers, appearance of a red-violet rim indicates presence of carbo-hydrates.

Fehling's Test - For this test, 1 mL of filtrate was heated in a water bath with 1 mL of each Fehling solution A (aqueous Copper Sulphate solution) and B (aqueous Potassium Sodium Tartrate solution). The presence of carbohydrates is indi-cated by presence of a brick-red precipitate.

Detection of Cardiac Glycosides (Keller-Kiliani Test)

For the detection of cardenolides, around 0.5 g of extract was diluted in 2ml of glacial acetic acid, followed by addition of one drop of 1% ferric FeCl3 solution. This mixture was then thoroughly treated with sulfuric acid solution. The presence of a brownish ring at the interphase suggested the existence of deoxy sugar, a characteristic of cardenolides. An initial greenish ring could be observed just above brown ring within acetic acid layer, which then expanded across this layer. Meanwhile, a violet ring initially appeared below brown ring.

Saponin detection

For the Foam Test, extract was diluted to 20mL using distilled H2O & resultant solution wasvigorously shaken for 15 minutes in a graduated cylinder. The persistence of a foam layer about 1 cm thick for 15 minutes served as an indi-cation of presence of sapo-nins.

Detection of Proteins & Amino Acids

A sample of 100 mg of extract was dissolved in 10ml of distilled H2O & then passed through Whatman No. 1 filter paper. The resulting filtrate was sub-jected to following tests:

For the biuret test, a single drop of a 2% solution of copper sulfate was added to a 2 mL portion of the filtrate. This mixture was then treated with 1ml of 95% ethanol, followed by a few granules of KOH. The presence of proteins was suggested by the emergence of a pink color in the ethanol layer.

For the amino acid detection, 2-2 drops of a nin-hydrin solution (composed of 10mg of nin- hydrin in 200ml of acetone) were added to 2-milliliters of aqueous filtrate. The appearance of a pronounced purple color signaled presence of amino acids.

Phytosterol Detection (Libermann-Burchard's Test)

To ascertain presence of phyto-sterols in the extract, 50mg of sample was solubilized in 2 ml of acetic anhydride. Subsequently, a few drops of conc. H2SO4 were carefully introduced down side of test tube. The emergence of a brown ring at layer intersection, as well as a green color in top layer, pointed towards existence of phyto-sterols.

Tannin and Phenolic Compound Detection

The FeCl3 test was utilized for identifying tannins and phenolic compounds. Here, 50mg ofextract was dissolved in 5ML of distilled H2O, followed by addition of a few drops of a 0.1% neutral fEcL3 solution. The presence of phenolic compounds & tannins was verified by emergence of a dark green shade or a blue-black coloration.

Alkaloids Detection

The detection of alkaloids was performed by mixing a few milliliters of diluted hydrochloric acid with 50 mg of the solvent-free extract, followed by filtration. Various tests were then conducted on the filtrate as follows:

Mayer's Test: A few drops of Mayer's reagent (a combination of mercuric chloride and potassium iodide) were introduced to 2mL of filtrate. The appearance of a white & creamy precipitate suggested a positive result.

Wagner's Test: A couple of drops of reagent (which contains potassium iodide) were addedto 2ml of filtrate. A reddish-brown precipitate signified a positive reaction.

Hager's Test: 1 or 2ml of reagent (aqs. solution saturated with picric acid) was added to 2mL of filtrate. The observation of a distinct yellow ppt signaled a positive outcome.

Dragendorff's Test: Between 1 to 2 ml of Dragendorff's reagent was introduced to 2 mL ofthe filtrate. A reddish-brown ppt suggested a positive test.

Terpenoids Detection (Salkowski Test): To detect terpenoids, extract was combined with 2ml of chloroform & concentrated H2 SO4to create a layered solution. The appearance of a reddish-brown hue at interface indicated presence of ter-penoids.

Quinones Detection
The process of detecting quinones involved combining the plant extract with several drops of sodium hydroxide, followed by vigorous shaking. The emergence of blue, green, or red shades served as an indication of the presence of quinones.

Flavonoids Detection
The identification of flavonoids was carried out by heating extract & combining it with an equivalent volume of ethanol, along with the addition of several drops of conc. HCl & a magnesium ribbon. The emergence of a red or pink hue served as an indication of flavonoidpresence.

Chromatographic Analysis
GCMS analysis: GCMS of the Oils will be performed outside the institute
Animals studies: The protocol the Institutional Animal Ethical Committee with the project. (1207/PO/Re/S/08/CPCSEA) dated 17 December 2019 and the approved for conducting theanimal activity.

Table 1: Indicating the Animals Grouping in the Experiment.

Group name	Group class	Treatment details
Group 1	Normal control	10 ml/kg distilled water (po)
Group 2	Disease control	10 ml/kg distilled water + Triton WR-1339 (IP)
Group 3	Std Group/Drug	Std. Drug (20 mg once a day.mg/kg po) + TritonWR-1339 (IP)
Group 4	Test 1	Extract I (200 mg/kg) + Triton WR-1339 (IP)
Group 5	Test 2	Extract I (400 mg/kg) + Triton WR-1339 (IP)
Group 6	Test 3	Extract I (200 mg/kg) + 200 (mg/kg) of Extract IIplus Triton 1339-WR(IP)

Abiochemical Evaluation
We measured total cholesterol (TC), tri-glycerides (TG), high-density lipo-protein cholesterol (HDL-C), low-density lipo-protein choles-terol (LDL-C), & very low-density lipo-protein choles-terol (VLDL-C) in serum extract using standard protocols.

Histopathological studies
Upon completion of the study, animals from the experimental group were humanely euthanized, with their heart and liver tissues collected for further examination. Standard procedures were applied to create cross sections of these tissues, which were then studied for any cellular changes suggestive of histopathology. These histopathological evaluationsof the heart and liver were conducted using a standard light microscope.

Statistical analysis
Mean S.E.M. was the format used to describe the findings. Analysis of Variance (ANOVA) & Dunnet's multiple comparison tests were used to establish statistical signi-ficance, with a p-value of less than 0.05 being deemed signi-ficant. GraphPad PRISM, version 5.0, was used for statistical analysis.

RESULTS AND DISCUSSION
Extraction of Oil: The plant matter, amounting to 2 kg each from Syzygium cumini and H.annus, was subjected to a series of processing steps. The plants were first air-dried, broken down into smaller fragments, dried again, and then coarsely pulverized. Following this, the plant materials underwent comprehensive cold compression without heat exposure, with subsequent testing performed on hydro extracts. Adopting a plant- focused diet, abundant in fruits, vegetables, and legumes, while being low in saturated fats, serves as a viable strategy for those with advanced atherosclerosis. This notion has been supported by research such as the study by Gao et al., 2002.

Table 2: Properties of Seeds of *Syzygium Cumini* and *H .Annus* Extract.

S.No	Properties	*Syzygium cumini*	*H .annus*
1	Type	Hydro alcoholic(1:1)	Hydro alcoholic(1:1)
2	Taste	Bitter	sweet
3	Color	Dark – yellow	Dark brown
4	% Yield	1.14%	1.95%)

Table 3: Indicating the Moisture Content in *Syzygium Cumini.*

Moisture Content		
S. No	Name of the sample	Percentage
1	*Syzygium cumini 1*	3.13
2	*Syzygium cumini 2*	3.52
3	*Syzygium cumini 3*	3.65
Mean (n=3)	3.43	
SD	±0.109	

Table 4: Indicating the Moisture Content in *H. Annus.*

Moisture Content		
S. No	**Name of the sample**	**Percentage**
1	***H .annus 1***	3.19
2	***H .annus 2***	3.46
3	***H .annus 3***	3.53
Mean (n=3)	3.56	
SD	±0.11	

Table 5: Indicating Foreign Matter Values in *Syzygium Cumini* Crude Drug.

S. No	Sample name	Percentage
1	***H .annus 1***	0.13
2	***H .annus 2***	0.18
3	***H .annus 3***	0.23
Mean (n=3)	0.18	
SD	±0.94	

Table 6: Indicating Total Ash values of *Syzygium Cumini.*

S. No	Sample name	Percentage
1	***Syzygium cumini -1***	4.2
2	***Syzygium cumini -2***	4.1
3	***Syzygium cumini -3***	3.1
Mean(n=3)	3.8	
SD	±0.7	

Table 7: Indicating Total Ash values of *H. Annus.*

S. No	Sample name	Percentage
1	***H.annus-1***	5.1
2	***H.annus-2***	5.2
3	***H.annus-3***	5.2
Mean(n=3)	5.16	
SD	±0.8	

Table 8: Indicating Total Ash values of *Syzygium Cumini.*

S. No	Sample name	Percentage
1	***Syzygium cumini-1***	0.98
2	***Syzygium cumini-2***	1.2
3	***Syzygium cumini-3***	1.0
Mean(n=3)	0.994	
SD	±0.1041	

Table 9: Indicating Water Insoluble Ash values of *H. Annus.*

S. No	Sample name	Percentage
1	***H .annus-1***	0.87
2	***H .annus 2***	1.3
3	***H .annus-3***	1.02
Mean(n=3)	2.4	
SD	±0.11	

Table 10: Indicating acid insoluble of *Syzygium Cumini.*

S. No	Sample name	Percentage
1	***Syzygium cumini -1***	0.04
2	***Syzygium cumini -2***	0.1
3	***Syzygium cumini -3***	0.06
Mean(n=3)	0.067	
SD	±0.029	

Table 11: Indicating acid insoluble of *H. Annus.*

S. No	Sample name	Percentage
1	***H .annus-1***	0.03
2	***H .annus 2***	0.2
3	***H .annus-3***	0.05
Mean(n=3)	0.057	
SD	±0.039	

Table 12: Indicating Water Soluble Extractives Values of *Syzygium Cumini.*

S. No	Sample name	Percentage
1	***Syzygium cumini -1***	2.4
2	***Syzygium cumini -2***	3.43
3	***Syzygium cumini -3***	3.2
Mean(n=3)	2.98	
SD	±0.56	

Table 13: Indicating Water Soluble Extractives Values of *H. Annus.*

S. No	Sample name	Percentage
1	***H .annus-1***	2.8
2	***H .annus 2***	3.29
3	***H .annus-3***	3.34
Mean(n=3)	3.20	
SD	±0.47	

Table 14: Indicating Methanol Soluble Extractives Values *Syzygium Cumini.*

S. No	Sample name	Percentage
1	***Syzygium cumini -1***	2.34
2	***Syzygium cumini -2***	2.62
3	***Syzygium cumini -3***	2.88
Mean(n=3)	3.21	
SD	±1.87	

Table 15: Indicating Methanol Soluble Extractives Values *H. annus.*

S. No	Sample name	Percentage
1	***H .annus-1***	4.32
2	***H .annus 2***	4.62
3	***H .annus-3***	4.83
Mean(n=3)	5.32	
SD	±1.23	

Table 16: Phytochemical Screening of Oils of *Syzygium Cumini and H. Annus.*

S. No	PhytochemicalConstituents	Tests/ Reagents	Hydro - alcoholic Extract *Syzygiumcumini*	HydroalcohlicExtract *H .annus*
1	Carbohydrates	Molish's reagentFehling's test	Absent Absent	Present Present
2	Tannins/Phenolic Compounds	Ferric chloride testLead acetate	Present Present	Present Present
3	Flavonoids	Shinoda test	Present	Present
4	Saponins	Foam test	Present	Absent
5	Alkaloids	Mayer's reagent Dragendorff's Reagent Hager's reagent Wagner's reagent	Present Present Present Present	Present Present Present Present
6	Proteins	Biuret's test Ninhydrin's test	Absent Absent	Present Present
7	Quinones	with NaOH	Present	Absent
8	Terpenoids	Salkowski test Sulphuric acidtest	Present Present	Present Present

9	Cardiac Glycosides	Keller-Killiani test	Absent	Present
10	Steroids	Liberman Burcha red test	Absent	Present

Table 17: Effect of *Syzygium Cumini and H. annus* on Body Weight.

Day	Normal Control	DiseaseControl	Atorvastatin 20mg/kg	H.A and SC (0.25+0.25)ml /kg	H.A and SC (0.50 +0.50) ml/kg	H.A and SC (0.75 +0.75) ml /kg
Day0	190.83±4.07	192.50± 5.01	191.17± 4.07	189.33±4.76	192.00±5.10	189.33±4.27
Day7	202.85±3.97	218.30± 4.11	217.27± 3.93	245.13±4.86	233.10±5.20	251.23± 4.34
Day14	211.67±5.11	334.33± 15.65	257.00± 10.00	273.14±13.23	269.83±13.98	266.17± 14.25
Day21	232.67±5.35	348.23± 16.61	277.80± 11.31	293.12±12.26	299.83±7.96	296.12± 13.23

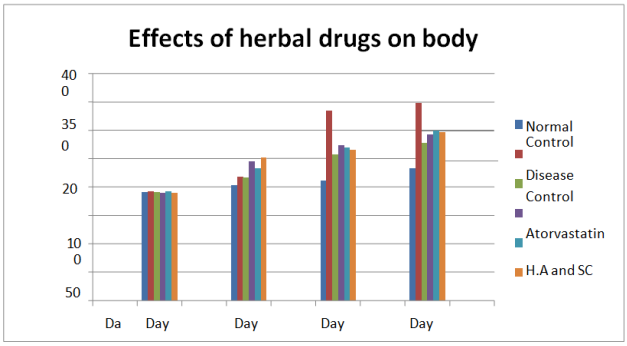
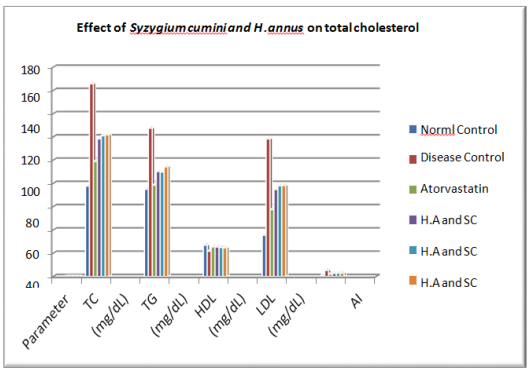


Figure 1: indicating the effects of treatments on body weight.

Table 18: Effect of *Syzygium Cumini and H. Annus* on Total Cholesterol.

Para-meter	Normal Control	DiseaseControl	Atorva statin 20mg/kg	H.A and SC 0.25ml /kg+ 0.25ml/kg	H.A and SC 0.50ml/kg +0.50ml/kg	H.A and SC 0.75 ml/kg +0.75 ml/kg
TC(mg/dL)	77.76±4.19	165.87±3.64	98.91± 4.59	118.37±3.23	121.13±3.05	121.98±4.59
TG(mg/dL)	75.07±4.24	127.62±7.21	78.63±3.46	90.24±4.43	89.83± 4.57	94.31±3.89
HDL(mg/dL)	27.08±1.23	21.92±0.89	25.62±1.03	25.48±0.66	25.08±0.43	24.81 ±0.37
LDL(mg/dL)	35.66±4.14	118.43±4.08	57.58±4.59	74.84±3.13	78.09±3.48	78.30±4.63
AI	1.32±0.19	5.41±0.35	2.25±0.18	2.94±0.17	3.11±0.14	3.16±0.19

Values are mean± S.D(n=6), p values:.(byone-way ANOVA followed by Dunnett multiplecomparison test)



Figur 2: Showing Effect of *Syzygium Cumini and H. Annus* on Total Cholesterol.

Table 19: Effect of *Syzygium Cumini and H. Annus* on Lipid Peroxidation.

Parameter	NormalControl	DiseaseControl	Atorvastatin 20mg/kg	H.A and SC 0.25ml /kg+0.25ml /kg	H.A and SC 0.50 ml/kg +0.50 ml/kg	H.A and SC 0.75 ml /kg+0 .75 ml /kg
LPO	3.93± 0.35[q]	7.53± 0.47[b]	3.84± 0.14[q]	5.46± 0.32	5.53± 0.48	5.58±0.41

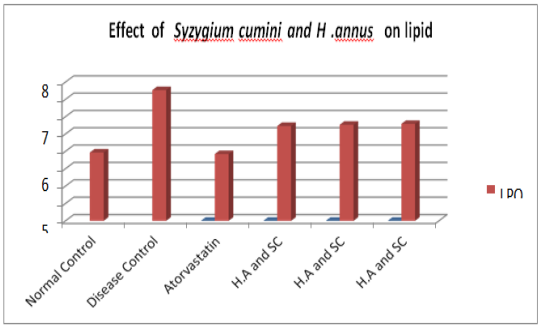


Figure 3: Showing Effect of *Syzygium Cumini and H. Annus* on Lipid Peroxidation.

Table 20: Effect of *Syzygium Cumini and H. Annus* on Hepatic Enzymes in Diet-InductedHyperlipidemic Rats.

Parameter	Normal Control	Disease Control	Atorvastatin 20mg/kg	H.A and SC 0.25ml/kg +0.25ml/kg	H.A and SC 0.50ml/kg +0.50ml/kg	H.A andSC 0.75 ml /kg+0.75ml /kg
ALP(U/L)	211.0 ±10.07	507.7 ±35.42	321.1 ±6.83	302.22 ±8.54	432.40 ±61.40	287.80 ±6.69
ALT(U/L)	36.15 ±3.71	75.61 ±8.41	50.82 ±.25	52.57 ±4.25	67.94 ±7.24	48.96 ±4.92
AST(U/L)	59.13 ±7.76	96.47 ±7.29	71.08±4.86	71.95 ±5.39	89.78 ±7.24	66.21 ±7.34

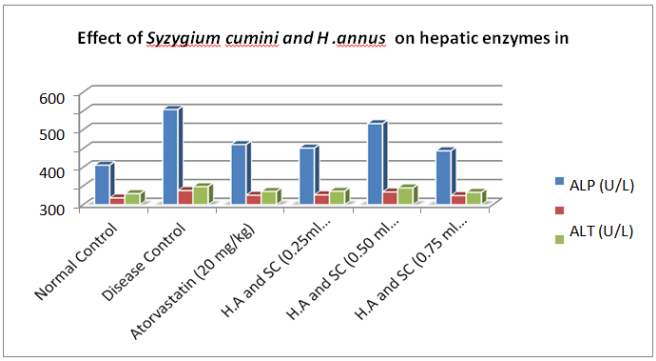


Figure 4: Showing the effect of *Syzygium cumini and H .annus* on hepatic enzymes in diet-inducted hyperlipidemic rats.

CONCLUSION

This pioneering study reveals the hypolipidemic properties of the medicinal herbs Helianthus annus and Syzygium cumin, potentially mitigating cardiovascular complications such as atherosclerosis. Administered at a dosage of 0.75 + 0.75 mlkg-1, these herbs were found to exert the most significant influence on cholesterol reduction. However, interestingly, they were also observed to have the minimal effect among all substances evaluated at the same dose. This lends supportto the need for additional investigations into the potential benefits of oil derived from seeds.

Polyphenols, potent antioxidants that can potentially impede the oxidation of low- density lipoprotein (LDL) cholesterol, deserve consideration. Even though the specific hypolipidemic mechanism remains to be elucidated, the favorable effects of polyphenols should not be overlooked. This necessitates further scientific scrutiny to uncover the exact process underlying these actions.

The combination of these selected herbal extracts demonstrated noteworthy hypolipidemic outcomes. Utilizing a Triton-induced rat model, the oils extracted

from these herbs were evaluated for their cholesterol-lowering abilities in blood. The findings provide an initial understanding of the potential benefits of these herbal extracts, paving the way for further research.

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