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RESEARCH ARTICLE
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PHYTOCHEMICAL ANALYSIS, ANTHELMINTIC AND ANTILIPIDEMIC ACTIVITIES OF *ILLICIUM VERUM* FRUIT CULTIVATED AT KARACHI, PAKISTAN

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ABSTRACT

Phytoextracts and phytochemicals are widely incorporated into medicine; hence, the scientific evaluation of herbs and plants for their effects on health is progressively increasing. Moreover, the use of herbal medicines has risen globally, as per the World Health Organization report of 2003, due to their easy availability, cost-effectiveness, and greater safety index. *Illicium verum* Hook. f., is commonly called Star Anise. Although it has been used as a spice and medicine since ancient times, the research data on the use of its fruit against certain common diseases or disorders is insufficient. This study was aimed at phytochemically analyzing the *Illicium verum* fruit grown in Karachi, Pakistan, and also evaluating its anthelmintic and anti-lipidemic activities. The phytochemical analysis of the aqueous extract of *Illicium verum* fruit revealed flavonoids, tannins, saponins, sterols, terpenoids, glycosides, phenolic compounds, and other bioactive constituents. Fecal egg reduction by 200 mg/kg/day dose was 75.5% and reductions in total cholesterol, low-density lipoprotein cholesterol, and triglycerides were 29.2%, 32.2%, and 39.5%, which were highest, followed by 150 mg/kg/day and 100 mg/kg/day doses. The increase in beneficial high-density lipoprotein cholesterol by 200 mg/kg/day dose was 50%. Significant dose-dependent anthelmintic and antilipidemic activities are exhibited by aqueous extract of *Illicium verum* fruit. Hence, in the future, the phytochemicals of *Illicium verum* fruit may be added to formulate novel drugs against helminthiasis and hyperlipidemia with an advantage of protection to the surrounding fauna and flora due to its organic nature, while considering the WHO guidelines for the monitoring of herbal medicines.

Keywords: Star anise, *Illicium verum*, Phytochemical analysis, Anthelmintic, Antilipidemic, Dose-dependant, Fruit extract, Fauna and flora, Pakistan, World Health Organization

INTRODUCTION

The scientific evaluation of herbs and plants for their effects on health is increasing day by day, because herbal medicines are still a preferred choice for the prevention and treatment of various diseases and disorders globally due to their organic nature, easy availability, affordability, and fewer side effects (Wachtel-Galor & Benzie, 2011). About one-fourth of the total prescribed medications are plant-based products worldwide (Sahoo et al., 2010). Not only from developing countries, but also 70% of the global population from developed countries depend on complementary and alternative medicines, including herbal medications. Hence, the World Health Organization developed the global traditional medicine strategy 2014-2023 and also the guidelines for the monitoring of herbal medications in terms of their safety and pharmacovigilance (WHO, 2013; WHO, 2004). Out of the total 591 drugs in the World Health Organization Model List of Essential Medicines 2023, more than 11% of the drugs are obtained from plants (WHO, 2023).

Haemonchus contortus is a common pathogenic nematode that causes more than ten million rupees of economic losses annually to livestock and farmers in Pakistan. This gastrointestinal parasite sucks blood, leading to anemia, decreased milk production, weight loss and death. The annual treatment cost of haemonchosis is worth approximately twenty-five million Pakistani rupees (Qamar et al., 2011). Anthelminthics are the drugs that are used to control and treat infections caused by parasitic worms, i.e. trematodes, cestodes and nematodes. Although anthelmintics have prime importance in veterinary and human medicine, but their unwanted penetration into plants, aquatic animals and soil gives rise to environmental contamination, toxic effects, decreased agricultural production and disturbed ecosystems. These negative effects are due to the physicochemical properties and synthetic chemical structure of anthelmintic drugs (Vokral et al., 2023).

Hyperlipidemia is characterized by an increase in low-density lipoprotein cholesterol, total cholesterol, and triglycerides, or/and decrease in high-density lipoprotein cholesterol in the blood due to abnormal metabolism of lipids (El-Tantawy & Temraz, 2019). Hyperlipidemia leads to oxidative stress, atherosclerosis and various other cardiovascular diseases in animals and humans (Al-Ezzy & Hameed, 2021). The medications for the management of dyslipidemia are life-long, hence phytotherapy or traditional herbal medicine are preferred due to their increased benefits, low toxicities, and higher therapeutic indices as compared to the lipid-lowering medicines that are obtained from synthetic sources (Kang et al., 2023).

Illicium verum Hook. f. is commonly called Star Anise. According to the APG IV system, it is classified under the family Schisandraceae and genus Illicium. Its tree is medium-sized, native to the southwest region of China and hence also referred to as the Chinese Star Anise. It is also cultivated in the tropical and subtropical regions of Asia. The fruit of *I. verum* plant is rust-colored, approximately 3cm in length, star-like with 5 to 10 pointed projections, and tough-skinned. Its fruit and seeds have been used as culinary spices and medicines since ancient times (Sharafan et al., 2022; Shahrajabian et al., 2019). The fruit of *I. verum* has been used to treat more than ten human diseases and disorders in Asia and North America. *I. verum* is a reservoir of more than fifty biologically active compounds, such as lignans, terpenes, flavonoids, and phenylpropanoids etc. In Traditional Chinese Medicine (TCM), *I. verum* fruit has been used to treat insomnia, rheumatism, inflammatory disorders of the skin, stomach aches, Qi flow regulation, antiemetic, control of cold, and analgesia. In the modern system of medicine, the active constituents and extracts of *I. verum* are used as antioxidant, anticonvulsant, insecticidal, sedative, anti-influenza, and antimicrobial (Wang et al., 2011).

The quality and chemical composition of a plant depend upon various factors such as soil, plant species, collection time, climate, storage conditions, altitude, processing, and endophytic organisms (Atanasov et al., 2015). Although much of the pharmacological research has been done, the established research data on *I. verum* fruit for its use against certain common diseases or disorders is still very scarce. Hence, this study aimed to phytochemically analyze the *Illicium verum* fruit grown at Karachi, Pakistan and also evaluate its anthelmintic and anti-lipidemic activities.

MATERIALS AND METHODS

Procurement and Recognition of *Illicium verum* fruit

The fruit of *I. verum* was purchased from an authentic herbal store in Karachi, Pakistan. It was identified and verified by a botanist and taxonomist from Herbarium and Botanic Garden, Center for Plant Conservation, University of Karachi.

Aqueous extract of *Illicium verum* fruit

The fruit of *I. verum* was gently washed and kept at 37˚C in a water bath for 24 hours with periodic stirring. It was then cut into small pieces and oven-dried at 45˚C. The material was ground to powder using mortar and pestle, and sieved through a 40-mesh sieve. The powder was weighed using a weighing balance. 250 gm of *I. verum* fruit powder was transferred to a volumetric flask and distilled water was added gradually with vigorous shaking. The volume was made up to 500 ml using the same solvent. This extract was filtered through Whatman grade no. 1 filter paper. The final concentration of the aqueous extract of *I. verum* fruit was 500 mg/ml or 0.5 gm/ml (Alhajj et al., 2020).

Experimental Animals

Experimental albino rabbits of either gender were procured after their thorough physical examination from Hussain Ebrahim Jamal (HEJ) Research Institute of Chemistry, University of Karachi. The animals were retained in plastic cages in the Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi. The environmental conditions were kept well-controlled. The temperature was maintained at 22 ± 2˚C while humidity was 50-60% on an alternative 12-hour light and dark cycle. A standard diet and sufficient water was provided for all the experimental animals. The guiding rules of the National Institute of Health Office of Laboratory Animal Welfare were followed throughout the study for handling as well as experimentation of animals. Animal Welfare Act 7 U.S.C 2131 and United States Federal policies, laws, and guidance for care, use, and transportation of animals were focused (NIHOLAW, 2011). This study was conducted after the approval of the Advanced Studies and Research Board (ASRB), University of Karachi.

A) Phytochemical Screening of *Illicium verum* fruit

Following tests were performed for the phytochemical screening of *I. verum* fruit (Banu & Cathrine, 2015, Sharma et al., 2020),

Frothing Test

1 ml aqueous extract of *I. verum* fruit + 15 ml distilled water + Shaken vigorously for 15 minutes → Formation of stable layer of froth reveals saponins

Ferric Chloride Test

1 ml aqueous extract of *I. verum* fruit + 5 ml distilled water with shaking to dissolve the contents + 3 drops of 5% ferric chloride solution → Dark green color reveals tannins

Alkaline Reagent Test

1 ml aqueous extract of *I. verum* fruit + 3 drops of 10% ammonium hydroxide solution → Yellow fluorescence reveals flavonoids

Legal's Test

1 ml aqueous extract of *I. verum* fruit + 2 ml pyridine solution to dissolve the contents + 2 ml sodium nitroprusside solution + 3 drops of 10% sodium hydroxide solution → Pink color reveals glycosides

Mayer's Test
1 ml aqueous extract of *I. verum* fruit + 3 drops of mayer's reagent are poured along with the side wall of the test tube → Creamy white precipitates reveal alkaloids

Salkowski Test
5 ml aqueous extract of *I. verum* fruit+ 2 ml chloroform + 3 ml concentrated sulfuric acid poured along with the side wall of the test tube → Reddish brown color reveals terpenoids

Libermann-Burchard Test
1 ml aqueous extract of *I. verum* fruit + 2 ml acetic anhydride + 2 drops of concentrated sulfuric acid → Array of colors from red, blue to green reveal steroids/phytosterols

Gelatin Test
5 ml aqueous extract of *I. verum* fruit + 2 ml of 1% gelatin solution containing 10% sodium chloride → White precipitates reveal phenolic compounds

Ninhydrin Test
2 ml aqueous extract of *I. verum* fruit + 2 drops of ninhydrin solution (10 mg ninhydrin/200 ml acetone) → Purple color reveals proteins

Molisch's Test
2 ml aqueous extract of *I. verum* fruit + 2 drops of alcoholic α-naphthol solution with vigorous shaking + 3 drops of concentrated sulfuric acid poured along with the side wall of the test tube → Violet ring reveals carbohydrates

Saponification Test
1 ml aqueous extract of *I. verum* fruit + 3 drops of 0.5 N alcoholic solution of potassium hydroxide + 1 drop of phenolphthalein + heat for 2 hours in water bath → Soap formation reveals fats and fixed oils.

B) Anthelmintic Activity
Floatation Solution
180 gm sodium chloride salt (pickling salt) was weighed and transferred to a measuring beaker. Purified water was gradually added with continuous stirring and the solution is heated to dissolve the maximum quantity of salt to make a saturated solution. The solution was allowed to settle overnight. The final volume of the solution was 500 ml i.e., 0.00036 gm/ml or 0.36 mg/ml (Zajac and Conboy, 2012). Its specific gravity was 1.2.

Albendazole Suspension
10 ml of purified water was heated up to 90˚C. Sodium benzoate 0.2 gm was added to it and the mixture was cooled to attain 40˚C temperature. Citric acid 0.03 gm was dissolved in 5 ml of purified water separately and this solution was added to the sodium benzoate mixture. 0.15 gm xanthan gum was dispersed at 65˚C in 20 ml of purified water and the hydrated product was allowed to stand at room temperature. The solution and mixture were combined; 0.2 gm potassium sorbate and 19.5 gm sucrose were added with continuous stirring. 10 tablets of albendazole (200 mg each) were crushed to make a fine powder. 2 gm albendazole powder, 0.05 gm polysorbate 80, 6.5 gm sorbitol, and 0.012 gm simethicone were added to the already prepared solution. The solution was vigorously stirred, and the volume was up to 50 ml with purified water. The final volume of the albendazole suspension was 0.4 gm/ml or 40 mg/ml. The suspension was stored in an airtight light-resistant glass bottle at room temperature (Allen, 2018).

Procedure

The anthelmintic activity was determined by the Fecal Egg Count Reduction Test (FERCT) using McMaster technique. Fifty albino rabbits of either gender weighing 3-5 kg were divided into five groups that were labeled as control, standard, treatment group 1, treatment group 2, and treatment group 3 with ten rabbits in each group. The rabbits were prepared to infest *Haemonchus contortus* nematodes larvae. An average of 5000 larvae per rabbit was the induction dose to acquire gastrointestinal infection. Their fecal samples were collected after three weeks and that was considered pre-treatment day 0 (Alowanou et al., 2021). The control group was given distilled water 1 ml/kg/day in divided doses throughout the experiment. The standard group was given albendazole suspension 30 mg/kg/day at 12 hours' intervals for 28 days (YaoQian et al., 2013). Treatment group 1 was given 100 mg/kg/day dose, treatment group 2 was given 150 mg/kg/day dose, and treatment group 3 was given 200 mg/kg/day dose of aqueous extract of *I. verum* fruit (Shaikh, 2020). Fecal samples from each rabbit were collected post-treatment on day 3, 7, 14, 21, and 28 respectively. 2 gm feces and 28 ml floatation solution were mixed together. The eggs of parasites in feces were separated from debris due to the differences in their densities when mixed with the floatation fluid. Those parasitic eggs floated to the upper surface while the remaining debris settled down in the counting chamber. A small quantity of this upper surface fluid was placed through a transfer pipette on the McMaster slide that contained grid. The slide dimensions were 2.5 cm*7.5 cm and the grid size was 1 cm*1 cm (Kryon Labs, South Africa). The whole chamber was filled with even distribution of fluid on the slide. The grid eased the counting of parasitic eggs. The floatation time in the chamber was 3 minutes. This slide was mounted on the microscope and the grid lines were focused. Using a 10X objective, the numbers of eggs were counted. The calculations were done to evaluate the number of eggs per gram (EPG) in the feces (Zajac and Conboy, 2012; Al-Shaibani et al., 2009).

Eggs per gram (EPG) = (Eggs in chamber 1 + Eggs in chamber 2)

The percentage of fecal egg count reduction (FECR) was calculated by:

$\% FECR = a - b / a \times 100$

Where,

a = EPG pre-treatment

b = EPG post-treatment

C) Antilipidemic Activity

Atorvastatin Suspension

Ten tablets of atorvastatin calcium (40 mg each) were crushed in a mortar and pestle. 0.3 gm veegum, 1.5 gm xanthan gum, 0.8 gm aspartame, 0.3 gm saccharine sodium, and 2 drops of peppermint oil were added in 0.4 gm atorvastatin calcium powder. 50 ml purified water was added with continuous mixing. The volume was made up to 100 ml with purified water. The final volume of atorvastatin suspension was 4mg/ml or 0.04 gm/ml. The suspension was stored in an amber colored glass bottle at room temperature (Zaid et al., 2017).

High Fat Diet

A High fat diet (HFD) was prepared by combining 2% w/w cholesterol, 0.5% w/w cholic acid, and 5% w/w butter fat. This is also called Atherogenic diet (Siddiqi et al., 2012).

Procedure

Sixty albino rabbits of either gender weighing 2-3 kg were divided into six groups, i.e. normal control, hyperlipidemic control, standard, and treatment group 1, treatment group 2, and treatment group 3 with ten rabbits in each group. To induce hyperlipidemia, a mixture of a high fat diet and a normal diet was given to the hyperlipidemic control, standard, and treatment groups of rabbits at a dose of 0.5 gm/kg/day for 6 weeks. The normal control group of rabbits was given only normal diet and distilled water 1ml/kg/day in divided doses throughout the experiment. The standard group of rabbits was given atorvastatin suspension 10 mg/kg/day at 24 hours' intervals for 6 weeks after induction

(Sharma and Choudhary, 2014). Treatment group 1 was given 100mg/kg/day dose, treatment group 2 was given 150 mg/kg/day dose, and treatment group 3 was given 200 mg/kg/day dose of *I. verum* fruit extract after induction for 6 weeks (Shaikh, 2020). The rabbits were fasted for a period of 16 hours after the last day of the 6^{th} week. The blood was collected from the tail vein of each rabbit by a saphenous venipuncture method (Abatan et al., 2008). Blood samples were centrifuged for about 10 minutes at a speed of 3000 rpm to separate serum. The lipid profile including triglycerides, total cholesterol, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol levels were determined by using commercial test kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) and the results were recorded (Siddiqi et al., 2012). The percentage of decrease or increase in lipid profile parameters of the standard and treatment groups of albino rabbits was calculated.

Statistical Analysis

Through Statistical Package for Social Sciences software (SPSS) version 17, the student's t-test was applied, and statistical analysis was carried-out in this study. P-value less than 0.05 was considered significant ($P<0.05$).

RESULTS

A) Qualitative analysis of *Illicium verum* fruit

The qualitative analysis of aqueous extract *I. verum* fruit grown in Karachi, Pakistan revealed the presence of various biologically active phytochemicals.

Table 1: Phytochemical investigation of aqueous extract of *I. verum* fruit

Phytochemical Constituents	Present(+) / Absent (-)
Saponins	+
Tannins	+
Flavonoids	+
Glycosides	+
Alkaloids	+
Terpenoids	+
Steroids	+
Sterols	+
Phenolic compounds	+
Proteins	+
Carbohydrates	+
Fats	+

B) Fecal Egg Count Reduction

Figure 1 demonstrates the effects of different doses of *I. verum* fruit extract and albendazole on fecal egg count reduction of albino rabbits from day 0 to day 28 as determined by McMaster floatation technique. When compared to the control group, the standard and treatment groups of albino rabbits exhibited a noteworthy decrease in fecal egg count per gram, i.e. $P<0.05$ ($P=0.02$ for standard group, $P=0.03$ for treatment groups). There was no significant difference between the decreases in fecal egg count per gram of standard and treatment groups of albino rabbits, i.e. $P>0.05$, which revealed that the anthelmintic or nematicidal activities of albendazole and different doses of aqueous extract of *I. verum* fruit (100 mg/kg/day, 150 mg/kg/day, and 200 mg/kg/day) were almost similar.

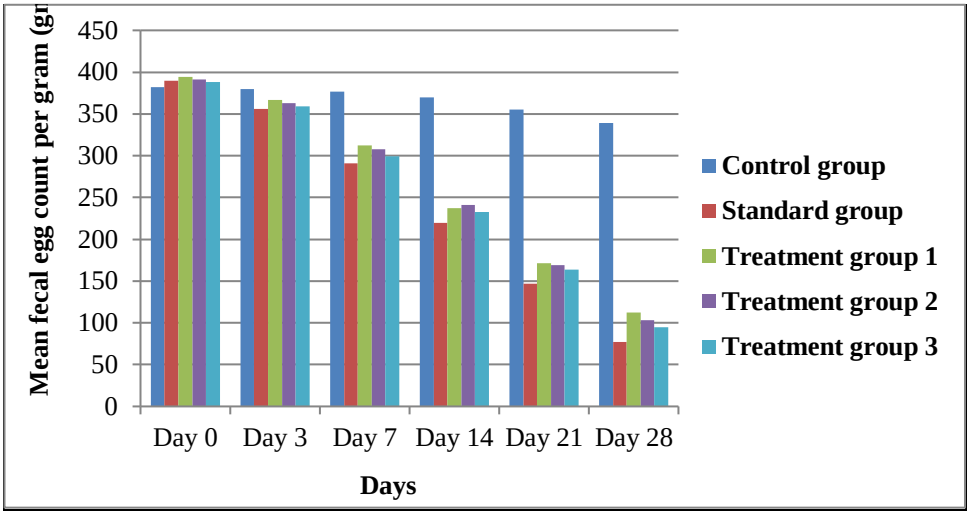
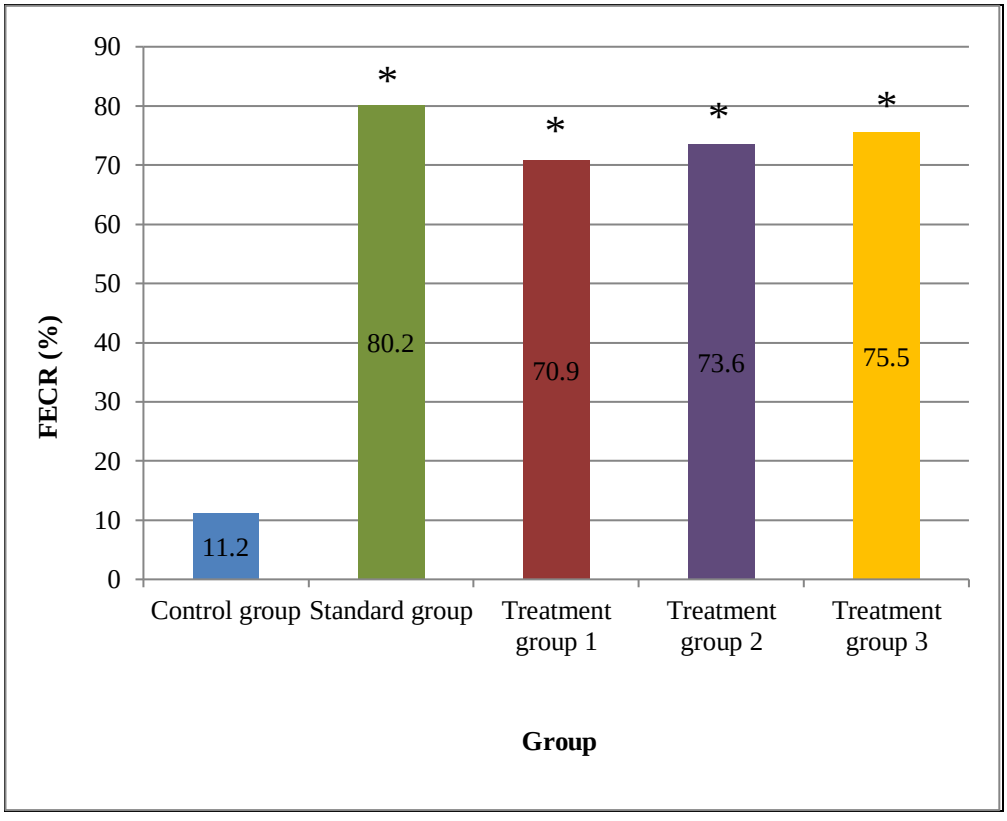


Figure 1. The effects of aqueous extract of *I. verum* fruit and albendazole on mean fecal Egg Count per Gram (EPG) of albino rabbits from day 0 to day 28

Percentage of Fecal Egg Count Reduction

Figure 2 demonstrates the overall percentage of fecal egg count reduction in standard and treatment groups of albino rabbits as determined by the average egg count per gram values in the pre-treatment and post-treatment phases. The percentage of fecal egg count reduction of the standard group of albino rabbits was highest, followed by the treatment group 3, 2, and 1. The albino rabbits that were given a 200 mg/kg/day dose of *I. verum* extract exhibited a 75.5% reduction in fecal egg count per gram. 73.6% reduction in fecal egg count per gram was exhibited by albino rabbits that were given 150 mg/kg/day dose of *I. verum* extract, while 71.5% reduction of fecal egg count per gram was revealed by albino rabbits that were given 100 mg/kg/day dose of *I. verum* extract. This showed a direct proportional relationship or a dose-dependent increase in the anthelmintic activity of aqueous extract of *I. verum* fruit.

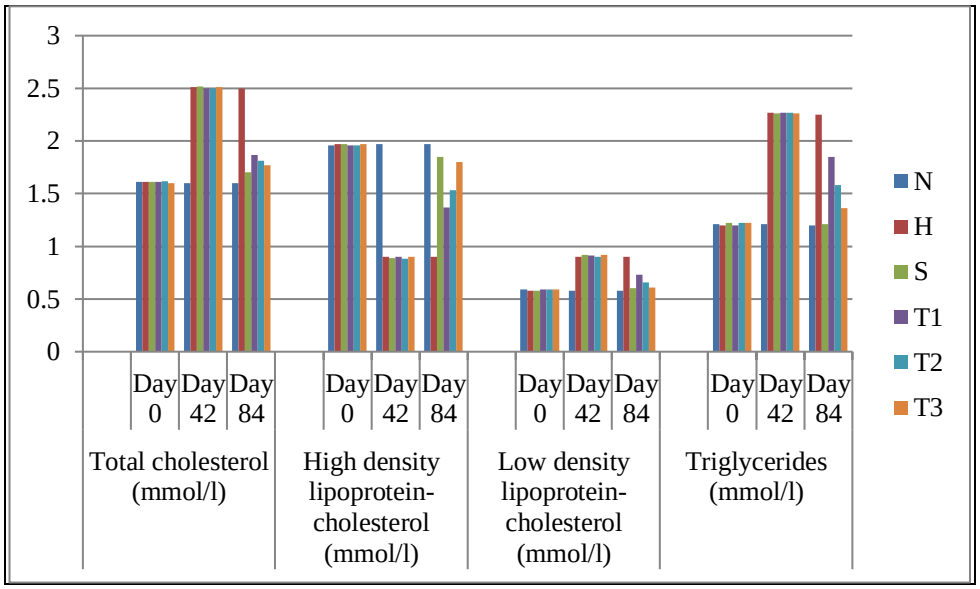


*P<0.05, significant reduction in fecal egg count as compared to control group of albino rabbits
Figure 2. The percentage of fecal egg count reduction in standard and treatment groups of albino rabbits

C) Lipid Profile

Figure 3 demonstrates the anti-hyperlipidemic effects of different doses of aqueous extract of *I. verum* fruit (100 mg/kg/day, 150 mg/kg/day, and 200 mg/kg/day) and the standard drug atorvastatin suspension 10 mg/kg/day on albino rabbits.

It resulted in significant reduction in the levels of total cholesterol, low-density lipoprotein cholesterol, and triglycerides, while an increase in high-density lipoprotein cholesterol of standard and treatment groups in contrast to the hyperlipidemic control group within six weeks (P<0.05).



N=Normal Control group, H=Hyperlipidemic Control group, S=Standard group, T1=Treatment group 1, T2=Treatment group 2, T3=Treatment group 3
Figure 3. The effects of *I. verum* fruit extract and atorvastatin on the lipid profile of albino rabbits

Total Cholesterol

When compared to the hyperlipidemic control group, the percentage of reduction in total cholesterol of the standard group of albino rabbits was 32%, while treatment group 1, 2, and 3 also showed significant reductions in total cholesterol within 6 weeks, i.e., 25.2%, 27.6%, and 29.2% respectively. Treatment group 3 exhibited the highest percentage of reduction in lipid profile parameters, followed by treatment group 2 and 1. With the increase in the dose of *I. verum* fruit extract, the levels of total cholesterol decreased.

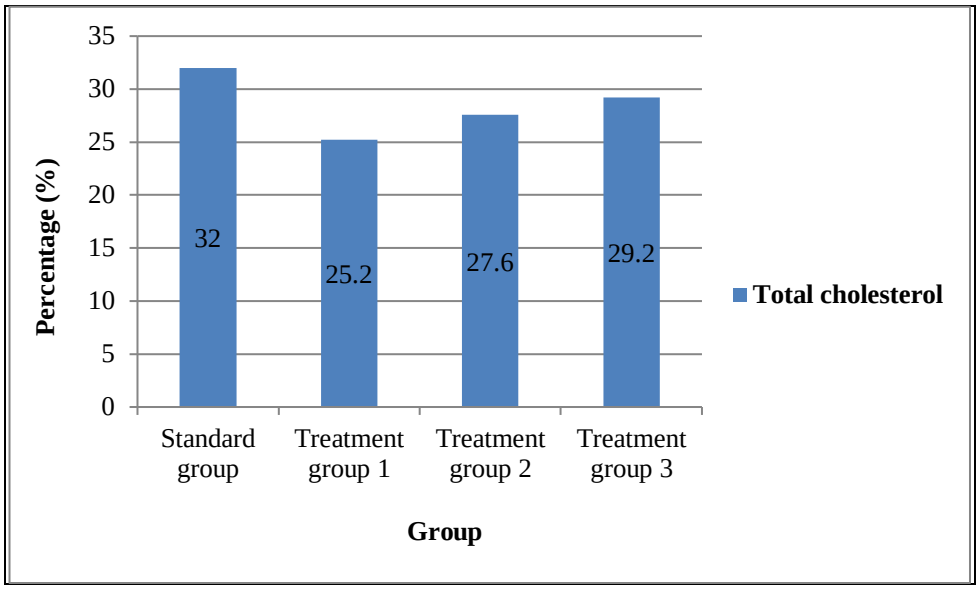


Figure 4. The percentage of decrease in total cholesterol of standard and treatment groups of albino rabbits

High-Density Lipoprotein Cholesterol

The percentage of increase in high-density lipoprotein cholesterol of the standard group of albino rabbits was 51.3%, while treatment group 1, 2, and 3 also showed significant increases in high-density lipoprotein cholesterol within 6 weeks, i.e., 34.3%, 41.1%, and 50% respectively when compared to hyperlipidemic control group. *I. verum* fruit extract revealed a dose-dependent increase in high-density lipoprotein cholesterol.

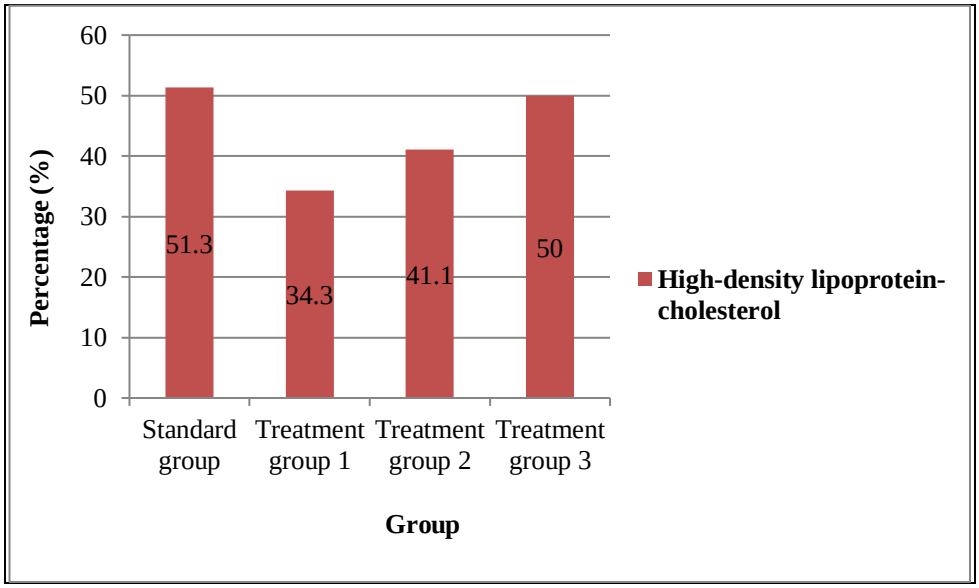


Figure 5. The percentage of increase in high-density lipoprotein cholesterol of standard and treatment groups of albino rabbits

Low-Density Lipoprotein Cholesterol

The percentage of reduction in low-density lipoprotein cholesterol of the standard group of albino rabbits was 33.3%, while there were also significant reductions in the low-density lipoprotein cholesterol levels of treatment group 1, 2, and 3 within 6 weeks, i.e., 18.8%, 26.6%, and 32.2% respectively when compared to hyperlipidemic control group. It showed that an inversely proportional relationship exists between the dose of *I. verum* fruit extract and levels of low-density lipoprotein cholesterol.

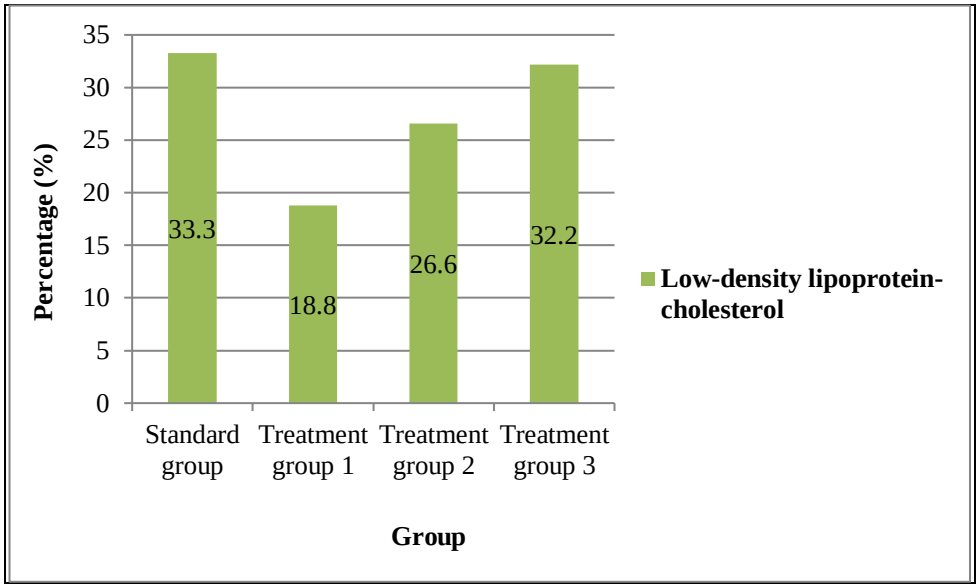


Figure 6. The percentage of decrease in low-density lipoprotein cholesterol of standard and treatment groups of albino rabbits

Triglycerides

The percentage of reduction in triglycerides of the standard group of albino rabbits was 46.2% within 6 weeks when compared to the hyperlipidemic control group. Treatment group 1, 2, and 3 showed dose-dependent reductions in triglycerides when compared to the hyperlipidemic control group, i.e., 17.7%, 29.7%, and 39.5% respectively. The levels of triglycerides decreased inversely with an increase in the dose of aqueous extract of *I. verum* fruit.

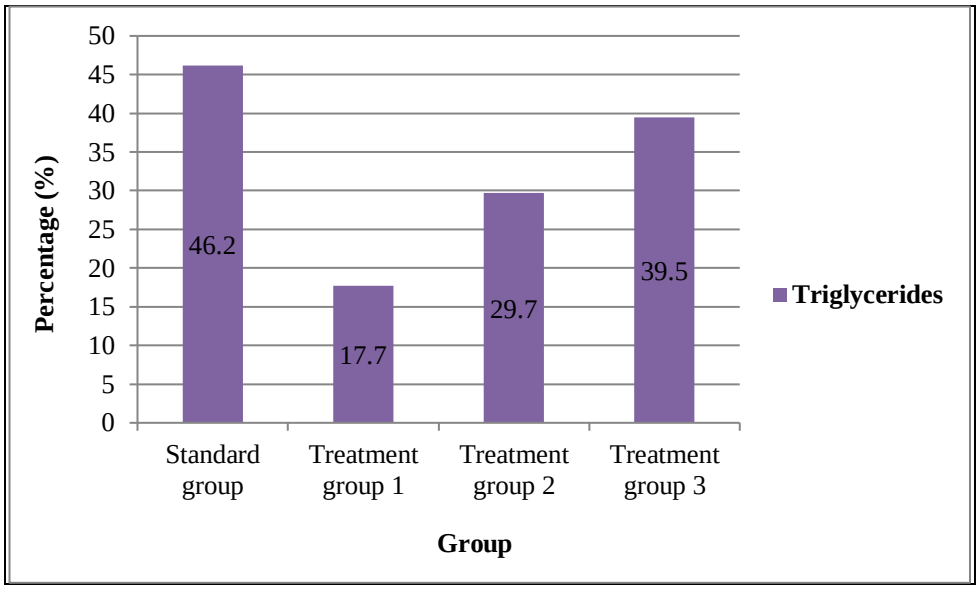


Figure 7. The percentage of decrease in triglycerides of standard and treated groups of albino rabbits

DISCUSSION

Illicium verum commonly called Star Anise, is an easily available, well-known herb that has been used for culinary and medicinal purposes since ancient times. The *Illicium verum* fruit that was cultivated in Karachi, Pakistan, revealed the presence of various biologically active phytochemicals including saponins, tannins, flavonoids, glycosides, alkaloids, terpenoids, steroids, sterols, and

phenolic compounds in its aqueous extract. Moreover, carbohydrates, proteins, and fats were also detected in it.

Anthelminthic drugs control and treat the infections caused by parasitic worms, i.e. trematodes, cestodes and nematodes. These parasitic worms are called Helminths and this infection is called Helminthiasis (Nixon et al., 2020). Parasitic nematodes infect plants, humans, and animals, leading to the deteriorated health of these organisms as well as a decrease in agricultural productivity. Hence, nematicides or anthelmintics are necessary to treat nematode infections along with the protection of the surrounding fauna and flora (Jasmer et al., 2003). The aqueous extract of *Illicium verum* fruit revealed dose-dependent anthelmintic activity when analyzed through a fecal egg count reduction test using the McMaster technique. The anthelmintic activity of the *I. verum* fruit is attributed to the tannins, flavonoids, and saponins contained in its aqueous extract. Tannins deformed the outer surfaces by binding with the collagens and other structural proteins on the cuticle of *H. contortus* larvae (Engstrom et al., 2016). Tannins affected egg hatching by blocking the exsheathment of *H. contortus* larvae along with a slight ovicidal effect (Klongsiriwet et al., 2015, Vargas-Magana et al., 2014). The quercetin flavonoid inhibited the production of energy by the blockade of phosphorylation reactions, altered the activities of stress-related enzymes, and generated oxidative stress in these nematodes. These events damaged their various organs and body parts, such as isthmus, pseudoceole, neurons, brut etc. The generation of ROS or free radicals paralyzed *H. contortus*. The death of these parasitic worms decreased the number of eggs in the feces of rabbits (Manjusa & Pradeep, 2022; Goel et al., 2023). The saponins increased the permeability of the membranes of *H. contortus* larvae and penetrated to destroy the contents inside them. They also interfered in their enzymatic activities that decreased the egg-hatching rate of these nematodes (Maestrini et al., 2019). The *H. contortus* nematode causes major problems for the agricultural industry, leading to economic losses. These nematodes can survive even in harsh environments and are difficult to eliminate (Sendow, 2003). The use of chemically synthesized anthelmintics can have harmful effects on the surrounding environment, such as plants, aquatic organisms, earthworms, fungi, and crops, because they are either excreted as toxic active metabolites or in their unchanged form (Haseler et al., 2024). The aqueous extract of *I. verum* fruit can be safely used as an anthelmintic because its organic nature will prevent plants, earthworms and other environmentally beneficial organisms from lethal effects.

Hyperlipidemia is characterized by an increase in plasma lipids, i.e., triglycerides, cholesterol, cholesterol esters, and phospholipids. Also, there is an increase in lipoproteins i.e., LDL (low-density lipoprotein) and VLDL (very low-density lipoprotein) and reduction in HDL (high-density lipoprotein) (Carpentier and Sobotka., 2008). The aqueous extract of *Illicium verum* fruit exhibited a dose-dependent decrease in total cholesterol, LDL-C (low-density lipoprotein cholesterol) and trigylcerides while a dose-dependent increase in HDL-C (high-density lipoprotein cholesterol) when analyzed upon high-fat diet fed albino rabbits. The HDL-C increasing efficiency of *I. verum* fruit extract is due to campesterol, polyphenols and phenolic acid contents, while total cholesterol, triglycerides, and LDL-C lowering efficiency are due to β-sitosterol, stigmasterol, polyphenols and phenolic acids. Plant sterols are widely known for their reducing activities on cholesterol, triglycerides, and low-density lipoprotein cholesterol in the plasma of hypercholesterolemia patients (He et al., 2018; Rideout et al., 2010, AbuMweis et al., 2014). The aqueous extract of *I. verum* fruit is rich in sterols such as β-sitosterol, campesterol, and stigmasterol. Of them, β-sitosterol is highest in quantity. 100 gm of star anise yields 256.9 mg β-sitosterol (Obranovic et al., 2020). β-sitosterol and stigmasterol are capable of reducing the elevated levels of cholesterol, total lipids, and triacylglycerols in the high-fat diet induced fatty liver. They also reduce fecal lipids and bile acids in the intestine. Stigmasterol prevents further increase in diacylglycerol, triacylglycerol, and phospholipids in the liver and blood (Feng et al., 2018). Phytosterols reduced the LDL-C levels in hyperlipidemic patients up to 12% or 0.3 mmol/L (AbuMweis et al., 2014). 2.5 gm per day consumption of plant sterols reduces approximately 10% serum LDL-cholesterol and also reduces the intestinal absorption of cholesterol (Smet et al., 2012; Jesch & Carr, 2017). 2% concentration of plant sterols can reduce 28% of plasma triglycerides within 6 weeks (Rideout et al., 2010).

Campesterol has a positive association with HDL-C levels (Stanasila et al., 2024). Star anise contains polyphenols and phenolic acids such as gallic acid, ferulic acid, chlorogenic acid, p-coumeric acid, and cinamic acid that possess DPPH radical scavenging efficiency up to 51.3%, hence reducing oxidative stress, VLDL, total cholesterol, LDL, and triglycerides while HDL increases (Iftikhar et al., 2022).

CONCLUSION

It is concluded by this study that the *Illicium verum* fruit grown in Karachi, Pakistan is rich in bioactive phytochemicals, including flavonoids, tannins, saponins, sterols, terpenoids, glycosides, and phenolic compounds etc. The aqueous extract of *I. verum* fruit decreases the elevated levels of total cholesterol, low-density lipoprotein cholesterol, and triglycerides, while increases the levels of beneficial high-density lipoprotein cholesterol. Moreover, the aqueous extract of *I. verum* fruit can be safely used as an anthelmintic against *Haemonchus contortus* nematode. Its organic nature provides an advantage of protection to the surrounding fauna and flora, unlike chemically synthesized anthelmintics. Hence, in the future, these bioactive phytochemicals of *I. verum* fruit may be incorporated into the formulation of novel drugs for the prevention and treatment of helminthiasis and hyperlipidemia, while considering the WHO guidelines for the monitoring of herbal medicines.

AUTHORS' CONTRIBUTIONS

DSS conceptualized, designed, conducted, and recorded the study; DSS analyzed the data and wrote the manuscript; SA provided technical assistance, supervised the work, and proofread the paper.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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