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Preliminary Phytochemical Analysis and Anti-Cholesterol Potential of Ethanolic Seed Extract of Illicium Verum

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Abstract

Illicium Verum commonly called star anise, which is indigenous to northeast Vietnam and Southwest China. Illicium verum is one of the vital ingredients of Chinese medicinal herbs. Principal component (Trans-anethol) exhibits antimicrobial activity such as antiviral, anti-bacterial, antifungal and antiparasitic activities. Cholesterol, a waxy or fat like substance which is found in each and every cell of our body. Cholesterol helps our metabolism work efficiently, but if our body has too much cholesterol in blood, it causes several harmful effects. Anti-cholesterol drugs are useful in reducing the blood cholesterol level. Ethanolic extract of Illicium verum was found to be rich in phytochemicals such as Alkaloids, terpenoids and steroids. The ethanolic extract of illicium verum exhibited a significant antioxidant potential (Ic50 = 300 µg/ml) and anti-cholesterol potential (Ic50 = 400 µg/ml) as compared to standard drugs.

Keywords*: Antimicrobial, Antiparasitic Activity, Anti-Cholesterol Drugs, Ethanolic Extraction, Innovative Technology, Novel Method, Star Anise.*

Introduction

Illicium Verum commonly called star anise, Chinese star anise, star aniseed, etc [1] 166 varieties and more than 42 species are present under the genus *Illicium verum*. *Illicium verum* belongs to the family Schisandraceae [2]. Distribution of this herb in Southwestern part of Asian continent [3]. This herb species originated from China and Vietnam [4]. This herb is used traditionally in China, Caribbean and by Latino populations [5]. *Illicium verum* is one of the vital ingredients of Chinese medicinal herbs [6] Principal component (Trans-anethol) exhibits antimicrobial activity such as antiviral, anti-bacterial, antifungal and antiparasitic activities [7]. It also possesses a number of other potentials such as secretolytic, anti-inflammatory, gastroprotective, etc [8]. Shikimic Acid, a major chemical component from star anise, which is the primary precursor for the synthesis of Oseltamivir (influenza drug) which is used as an antiviral medication for the treatment of influenza A and influenza B [9].

The fruit of *Illicium verum* Hook. f. (Chinese star anise) from a very long time used as a Chinese medicine and in the food industry [10] It is also used as a pain killer for relieving pain. essential oil extracted from *Illicium verum* containing anethole, used for flavoring drinks and confectionery [11]. Substances which neutralize free radicals contain antioxidant property. Scavenging of free radicals is of prime importance for protecting human health from disorders like cancer, degenerative and other diseases by counteracting with the mechanism of free radical formation [12]. Antioxidants help to prevent the harmful effects of free radicals by interrupting the mechanism of free radical formation and by neutralizing the free radical which leads to reduction of

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oxidative damage [13] Any agent that reduces the level of lipids and lipoproteins is considered a hypolipidemic drug. Petroleum ethers, chloroform, alcoholic and aqueous extract dose-dependently induce inhibition of cholesterol, triglycerides, and low-density lipoproteins level and significantly increase the high-density lipoprotein level.

Hypercholesterolemia majorly leads to heart diseases such as atherosclerosis, heart attacks, myocardial infarction, and cardiovascular disorders [14]. The risk factors that are separately or jointly responsible for the atherosclerosis and atherosclerotic lesions sequencing are hypercholesterolemia and hypertriglyceridemia [15]. Cholesterol leads to heart complications such as heart disease, stroke, and other vascular diseases. one-third of ischemic heart disease is caused due to increased cholesterol levels in the blood [16].

This study aims to evaluate the antioxidant and anti-cholesterol potential of ethanolic seed extract of *Illicium verum*.

Materials and Methods

Chemicals

All chemicals and reagents used for this research work were purchased from Sigma Chemical Company St. Louis, MO, USA; Invitrogen, USA; Eurofins Genomics India Pvt Ltd, Bangalore, India; New England Biolabs (NEB), USA.

Collection of Plant Material

The plant *Illicium Verum* was collected from Chennai District, Tamil Nadu, India. This species was identified and authenticated at the Department of Centre for Advanced Study in Botany, University of Madras, Chennai, India. Mainly the seed of this plant was dried, cut into small pieces and coarsely powdered. The coarse powder was used for extraction with ethanol.

Preparation of Plant Extracts

1kg of dry powders from seeds of *Illicium Verum* was taken in an aspirator bottle; 3 litres of ethanol was used, and the mixture was shaken occasionally for 72 hours. Then the extract was filtered. This procedure was repeated three times, and all extracts were decanted and pooled. The extract was filtered before drying using Whatman filter paper no 2 on a Buchner funnel and the solvent was removed by vacuum distillation in a rotary evaporator at 40˚C; the extract was placed in pre-weighed flasks before drying.

Phytochemical Screening Test

Test for Phlobatannin

1ml of the extract was treated with 1ml of 1% HCl and boiled for 10 mins. The formation of a red colour precipitate indicates the presence of phlobatannin.

Test for Carbohydrates

Three to five drops of Molisch reagent were added with 1 mL of the extract and then 1 mL of concentrated sulphuric acid was added carefully through the side of the test tube. The mixture was then allowed to stand for two minutes and diluted with 5 mL of distilled water. The development of a red or dull violet ring at the junction of the liquids showed the presence of carbohydrates.

Test for Flavonoids

A few drops of 1% liquid ammonia were taken in a test tube and along with it 1ml of the extract was added resulting in the formation of yellow color thereby indicating the presence of flavonoids.

Test for Alkaloids

2ml of the sample was mixed with 2ml of HCl. Then 6 drops of HCN were added and a further 2 drops of picric acid were added which resulted in a creamish pale yellow ppt indicating the presence of alkaloids.

Test for Terpenoids

2 ml of sample along with 2 ml of chloroform and 3 ml of con. H_2SO_4 was added.

Red color ppt obtained indicates the presence of terpenoids.

Test for Proteins

One milliliter of ninhydrin was dissolved in 1 mL of acetone and then a small amount of extract was added with ninhydrin. The formation of purple colour revealed the presence of protein.

Detection of Saponins

Foam test: A fraction of the extract was vigorously shaken with water and observed for persistent foam.

Test for Steroids

One ml of chloroform was mixed with 1 mL of extract and then ten drops of acetic anhydride and five drops of concentrated sulphuric acid were added and mixed. The formation of a dark red colour or dark pink colour indicates the presence of steroids.

Antioxidant Activity

DPPH Free Radical Scavenging Activity

Scavenging of 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radicals was assessed by the method of Hatano et al, (1989). DPPH solution (1.0 ml) was added to 1.0 ml of extract at different concentrations (0.1 to 0. 5mg/ml). The mixture was kept at room temperature for 50 minutes and the activity was measured at 517 nm. Ascorbic acid at the same concentrations was used as standard. The capability to scavenge the DPPH radical was calculated and expressed in percentage (%).

In Vitro Anti-Cholesterol Activity

The anti-cholesterol assay was carried out as described as per the kit method (Spinreact, S.A.U-Ctra Santa Coloma, Girona, Spain). Cholesterol was dissolved in chloroform at a concentration of 2.5 mg mL/ml. Ten microliters of the extract were pipetted into a microtiter plate followed by the addition of 2000 µL of R1 reagent and 10 µLof cholesterol as sample. Twenty microliters of distilled water and 2000 µL of R1 reagent were used as blank. Negative control consisted of 20 µL cholesterol and 2ml R1; standard consisted of 20 µL simvastatin and 2000 mL R1 reagent. The contents were incubated between 0-30 min at room temperature and the absorbance was read at 500 nm in a UV-Vis spectrophotometer against reagent blank. Anti-cholesterol assay of the extract was calculated.

Statistical Analysis

The data were subjected to statistical analysis using Two-way analysis of variance (ANOVA) and Tukey's multiple range test to assess the significance of individual variations between the groups. In Tukey's test, significance was considered at the level of $p<0.05$.

Result

Phytochemical Analysis of Ethanolic Extract of *Illicium Verum*

Table 1 describes that ethanolic seed extract of *Illicium verum* shows positive results for phytochemicals tests such as carbohydrates, alkaloids, proteins, steroids and terpenoids.

Table 1. Phytochemical Analysis of Ethanolic Extract of *Illicium Verum*

Phytochemicals	**Plant Name**	**Presence/Absence**
Carbohydrates	*Illicium verum*	+ **ve**
Saponins	*Illicium verum*	- **ve**
Alkaloids	*Illicium verum*	+ **ve**

Flavonoids	*Illicium verum*	-	**ve**
Proteins	*Illicium verum*	+	**ve**
Terpenoids	*Illicium verum*	+	**ve**
Steroids	*Illicium verum*	+	**ve**
Amino acids	*Illicium verum*	-	**ve**

Antioxidant Potential of Ethanolic Seed Extract of *Illicium Verum* Compared to the Standard (Vitamin C) by DPPH Radical Scavenging Assay

Fig 1 shows ethanolic extract of *Illicium verum* exhibiting a significant ($p<0.05$) antioxidant potential (Ic50 = 300 µg/ml) as compared to the standard drug vitamin C (Ic50 = 200 µg/ml).

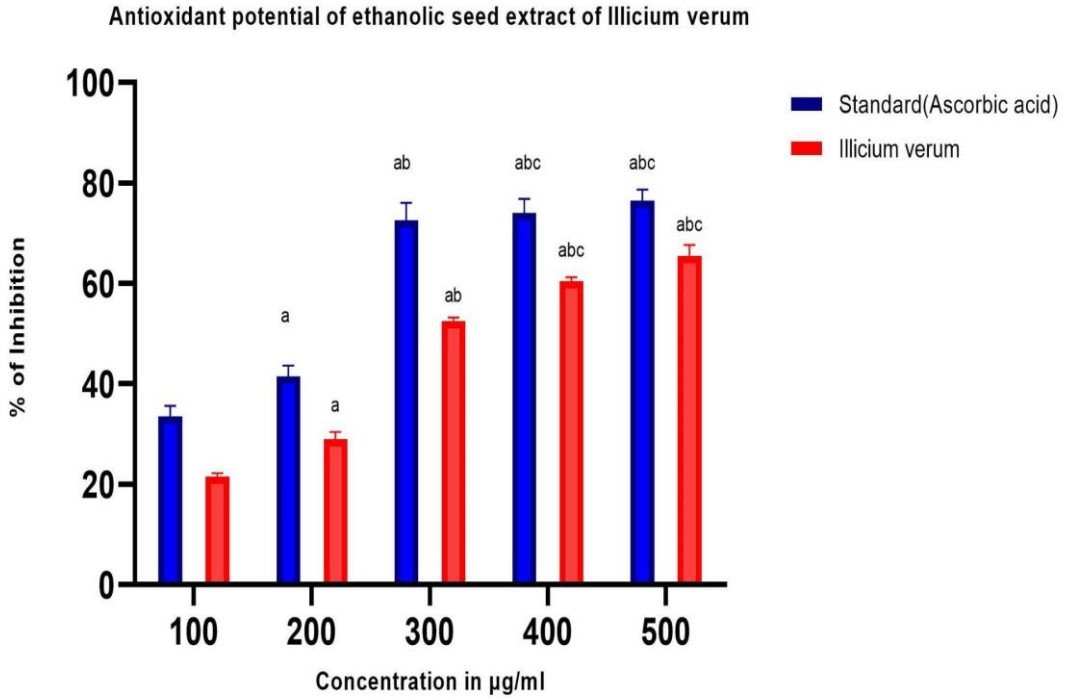

Figure 1. Antioxidant Potential of Ethanolic Seed Extract of *Illicium Verum*

Anticholesteremic Activity of Ethanolic Seed Extract of *Illicium Verum* Compared to the Standard (Vitamin C) by DPPH Radical Scavenging Assay

Fig 2 shows an ethanolic extract *of Illicium verum* exhibiting a significant ($p<0.05$) anti-cholesterol potential (Ic50 = 400 µg/ml) as compared to the standard drug simvastatin (Ic50 = 250 µg/ml). Both the antioxidant and anti-cholesterol potential of ethanolic seed extract of *Illicium verum* increased in a dose-dependent manner as compared with their standard drugs Vitamin C and Simvastatin.

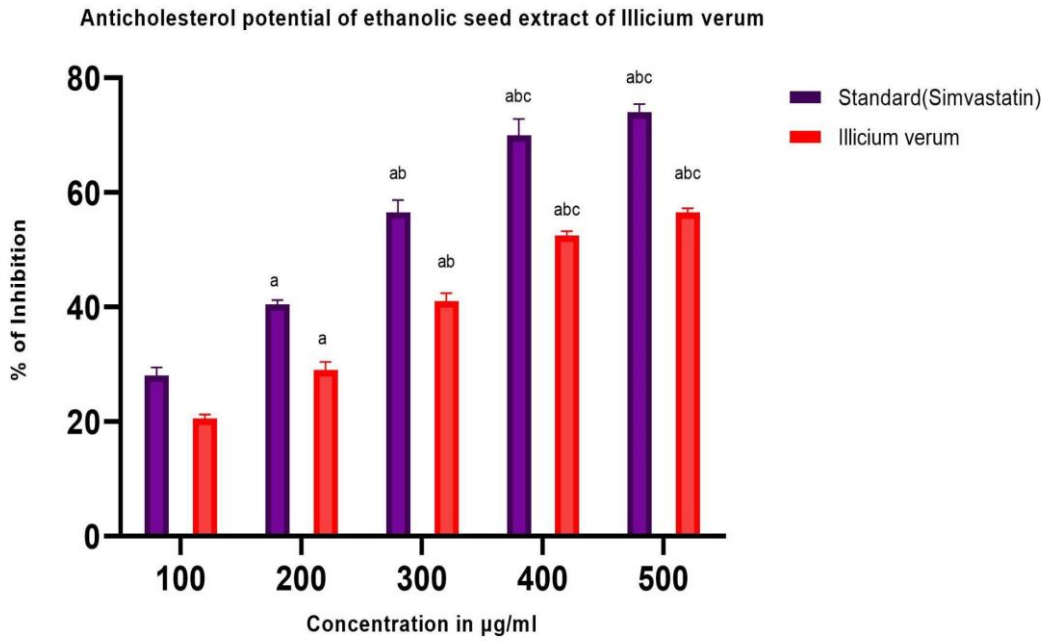

Figure 2. Anti-Cholesterol Potential of Ethanolic Seed Extract of *Illicium verum*

Discussion

The Ethanolic seed extract of *Illicium verum* was found to be rich in phytochemicals such as alkaloids, steroids and terpenoids which may be the reason for the good antioxidant potential of the extract. *Illicium verum*, being a spice, is found to be rich in aromatic phytonutrients such as alkaloids. In the study done by [17] it is found that the acetone extract of *Illicium verum* contains phytochemicals such as flavonoids, alkaloids, tri-terpenoids, tannins, steroids and cardiac glycosides, Ethyl acetate extract of *Illicium verum* contains phytochemicals such as alkaloids, tannins and steroids. In the previous study [18] it was found that Star anise extract is a low-toxic compound (LC50 = 212.09 ppm). Terpenoids are major antibacterial compounds present in star anise extract, and fatty acid and benzaldehyde are found as minor compounds. Thus, from the previous findings, it is understood that the phytoconstituents of an herb depend upon the nature of the solvent used for its extraction. In future a comparative study on the phytoconstituents with various solvents can be done.

Alkaloids, steroids and terpenoids help to fight the generation of free radicals. The antioxidant potential of ethanolic seed extract of *Illicium verum* increases in a dose-dependent manner. The ethanolic seed extract of *Illicium verum* was prepared and analysed for antioxidant activity by using DPPH (Free radical scavenging assay). The results obtained in the study show that the ethanolic seed extract of *Illicium verum* has significant antioxidant activity. In the previous study done by Benmalek et al, 2013, it was found that Flavonol has high antioxidant activity as compared to anthocyanins and standard antioxidants -ascorbic acid and quercetin. *A.cepa* and *C.oxyacantha ssp monogyna* exhibited effective antimicrobial activity, which may be due to their rich phytoconstituents [19]. From the study of it is found that ethanolic extract of *B. pinnatum* whole plant, *D. ovata* bark, *D. fortunei* rhizomes and *L. wallichii* barks inhibit the free radical in dose-dependent manner with the inhibition percentage at the concentration. Antioxidant plant drugs play an important role in the prevention and treatment of various disorders, caused by oxidative stress [20]. The antioxidant potential of a herbal extract is key for oxidising the free radicals which can lead to

disorders like inflammation, cancer, diabetes and atherosclerosis. From the current study, the significant antioxidant activity of the ethanolic extract of *Illicium verum is* promising.

The exact number was analysed for its anti-cholesterol activity. Simvastatin was a standard drug used for this study, which shows a positive control. The anti-cholesterol potential of ethanolic seed extract of *Illicium verum* compared to the standard (Simvastatin) is considered more significant because the standard drug simvastatin is usually preferred as the synthetic drugs which causes side effects such as muscle pain, and decreased platelet count though it is more efficient in decreasing cholesterol level than the herbal extracts. In a previous study [21] it was found that administration of *C. occidentalis extract* significantly ($p < 0.05$) prevented the elevation of TC, LDL-C, VLDL-C, hepatic and aortic TG and TC and treating with herbal extract leads to an increase in faecal cholesterol by decreasing the atherogenic, triglycerides, and lipid peroxidation (TBARS) index.

From the study [22] it is found that high cholesterol in rats' diet for eight weeks leads to increases of TC, TG, LDL-C, AST, ALT and MDA levels. Administration of ethyl acetate extract of *Mikania micrantha* stems (EAMS) and simvastatin significantly reduced the levels of TC, TG, LDL-C and MDA.EAMMS extract has anti-hypercholesterolemia properties. From the study [20] it is found that as compared with the anti-hyperlipidemic drug simvastatin (95.1%) ,maximum inhibition (93.04%) was noticed by observing the methanol in extract from flaxseed for 20 min, which indicates that methanol in an extract from flaxseed has a high anticholesterol activity to reduce or control the cholesterol levels.

The results obtained in the study show that ethanolic seed extract of Illicium verum has significant anticholesterol activity. Further, in vivo studies may be done to analyse the potential health benefits in the prevention and generation of reactive oxygen species and cholesterol-associated disorders.

Conclusion

Our body needs cholesterol to build healthy cells, but high levels of cholesterol can increase the risk of heart disease and can also develop fatty deposits in blood vessels. From the current study, it was proved that the ethanolic extract of *Illicium verum* possesses significant anti-cholesterol potential. Exploring the anti-cholesterol properties of herbs like *Illicium verum* is the need of the day, as these herbs are cost-free, easily available and part of the diet. Increased cholesterol and lipids pose a lot of health hazards. Nowadays more research is being done to explore the natural anti-cholesterol agents to decrease the potential risk caused due to hypercholesterolemia and synthetic drugs.

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Conflict of Interest

The author hereby declares that there is no conflict of interest in this study.

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