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Evaluation of Antioxidant and Xanthine Oxidase Inhibitory Potential of Ethanolic Seed Extract of Illicium Verum (Star Anise)

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Abstract

Illicium verum is a dark-coloured spice found in tropical areas of Asia. It is known for its medicinal value, flavoring properties and cosmetic properties. It helps in dispelling cold and relieving pain. Gout is a form of arthritis that is characterized by severe pain, redness and tenderness of joints. This is due to accumulation of uric acid crystal. Anti-gout activity of the herbal extract can be analyzed by its xanthine oxidase inhibitory potential.

Keywords: *Antioxidant, Illicium Verum, Innovative Technology, Novel Method, Uric Acid Crystals, Xanthine Oxidase Inhibitory Potential.*

Introduction

Illicium verum is a dark brown coloured spice found in tropical areas of Asia. It is known for its medicinal value, flavoring properties and cosmetic properties. It is used as a traditional medicine in China to treat cold and relieve pain. It is also proved to possess antimicrobial, sedative and analgesic properties. It is a major source for "shikimic acid" which is used in the formulation of antiflu drugs [1]. In the study done by [2], it was proved that methanolic seed extract of *Illicium verum* possesses antifungal activity as it inhibits the growth of all dermatophytic and saprophytic species. Antibacterial activity of *Illicium verum* (ethanolic extract) against 67 drug-resistant isolates such as *Acinetobacter baumannii, Pseudomonas aeruginosa and Staphylococcus aureus* was proved by [3].

Antioxidant activity of any herb is determined by its ability to scavenge free radicals released due to oxidative stress. Free radicals (FR) are produced through biochemical and physiological processes in the human body. Accumulation of free radicals might lead to oxidative damage of important biochemicals present in the body (proteins, lipids and DNA) which might lead to disorders such as cancer, diabetes mellitus, atherosclerosis etc. Herbal plants produce a wide range of secondary metabolites such as nitrogen compounds (alkaloids and amines) and phenolic compounds (phenolic acids, quinines, coumarins, flavonoids) that exhibit antioxidant activity. Among secondary metabolites produced by herbal plants, flavonoids are found to exhibit strong antioxidant activity [4].

Gout is a form of arthritis that is characterised by severe pain, redness and tenderness of joints, especially the first metatarsophalangeal (MTP) joint. This is due to accumulation of uric acid crystals (monosodium urate). Risk factors that can lead to the development of Gout can be hyperuricemia, dietary factors, alcohol addiction, genetic factors that lead to increased purine metabolism, chronic renal disease etc [5, 6]. To treat gout, the serum uric acid levels should be kept below 360 micromol/ml. The available medications to reduce serum uric acid levels are allopurinol, various uricosuric agents such as benzbromarone and non -steroidal anti-inflammatory drugs such as colchicine and

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glucocorticoids [7, 8]. However, allopurinol exhibits a wide range of side effects such as hypersensitivity, impaired renal function and also induces prostate cancer [9, 10]. Any herbal extract is said to possess antigout activity if it inhibits the activity of xanthine oxidase enzyme which induces purine metabolism[11]. Therefore, the aim of the study is to evaluate the xanthine oxidase inhibitory potential and antioxidant activity 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 *Illicium verum* seeds were collected from Chennai District, Tamil Nadu, India. The species were identified and authenticated at the Department of Centre for Advanced Study in Botany, University of Madras, Chennai, India. The seeds of the plant were shade-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 of seeds from the plants were taken in individual aspirator bottles; 3 liters 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 extracts were 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 extracts were 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 red color 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 red or dull violet ring at the junction of the liquids showed the presence of carbohydrates.

Test for Flavonoids

Few drops of 1% liquid ammonia were taken in a test tube and along with it1ml of the extract was added resulting in the formation of yellow color thereby indicating the presence of flavonoids.

Test for Alkaloids: 2ml of sample was mixed with 2ml of HCl. Then 6 drops of HCN were added and further 2 drops of picric acid were added that resulted in a creamish pale yellow precipitate indicating the presence of alkaloids.

Test for Terpenoids

2 ml of sample along with 2ml of chloroform and 3ml of con. H2SO4 was added. Red color precipitate obtained indicates the presence of terpenoids.

Test for Proteins

One milliliter of ninhydrin was dissolved in 1 mL of acetone and then 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 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) radical was assessed by the method of Hatano et al, (1989) [12]. 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 Xanthine Oxidase Inhibitory Activity of Ethanoic Seed Extract of *illicium verum*

In vitro Xanthine oxidase inhibitory of the extract was assessed as per the method of (Nguyen et al, 2004; Umamaheswari et al., 2007) [13, 14] Briefly, the assay mixture consisted of 1 ml of the fraction (0.1 to 0.5g/ml), 2.9 ml of phosphate buffer (pH 7.5) and 0.1 ml of xanthine oxidase enzyme solution (0.1 units/ml in phosphate buffer, pH 7.5), which was prepared immediately before use. After preincubation at 25◦C for 15 min, the reaction was initiated by the addition of 2 ml of the substrate solution (150 M xanthine in the same buffer). The assay mixture was incubated at 25°C for 30 min. The reaction was then stopped by the addition of 1 ml of 1N hydrochloric acid, and the absorbance was measured at 290 nm using a UV spectrophotometer. Allopurinol (0.1 to 0.5mg/ml), a known inhibitor of XO, was used as the positive control. One unit of XO is defined as the amount of enzyme required to produce 1 mmol of uric acid/min at 25°C.

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

From the study, it was evident that the ethanolic seed extract of *Illicium verum* was found to be rich in phytochemicals such as Alkaloids, terpenoids and saponins (Table 1).

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

Sr.no	Phytochemicals	*Illicium verum*
1	Carbohydrates	+
2	Saponins	_
3	Steroids	+

4	Alkaloids	+
5	Flavonoids	_
6	Terpenoids	+
7	Proteins	+
8	Amino acid	_

Phytochemicals are the secondary metabolites which act as a natural defence against free radicals and also possess a range of medicinal properties.

Antioxidant Analysis

Antioxidant analysis of the ethanolic seed extract of *Illicium verum* was analysed and compared with the standard vitamin- C. IC_{50} of the extract was found to be 370µ g/ ml (Figure 1. Antioxidant potential of the extract increased in a dose dependent manner as compared to the standard (Vitamin C).

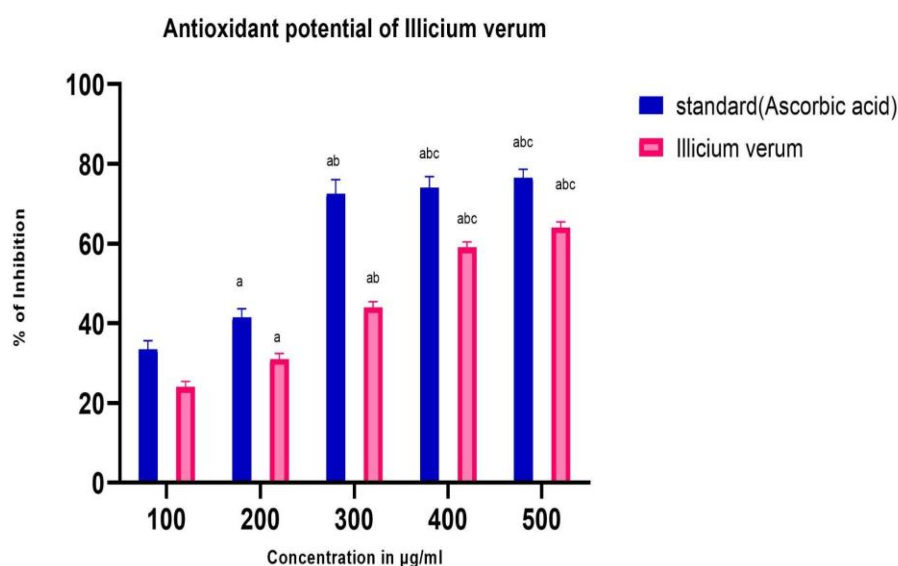

Figure 1. Antioxidant Potential of Ethanolic Seed Extract of Illicium Verum Compared with the Standard (Vitamin C)- Dpph Assay

***In vitro* Xanthine Oxidase Inhibitory Activity**

In vitro Xanthine oxidase inhibitory potential of the ethanolic seed extract of *Illicium verum* was assessed and compared with the standard Allopurinol. Anti-gout potential of the extract was analysed as a function of the extract to inhibit the enzyme Xanthine oxidase. The extract exhibited a significant anti gout potential (Figure 2) with an IC_{50} of 270µg/ml. Anti-gout potential of the extract increased in a

dose dependent manner as compared to the standard- Allopurinol.

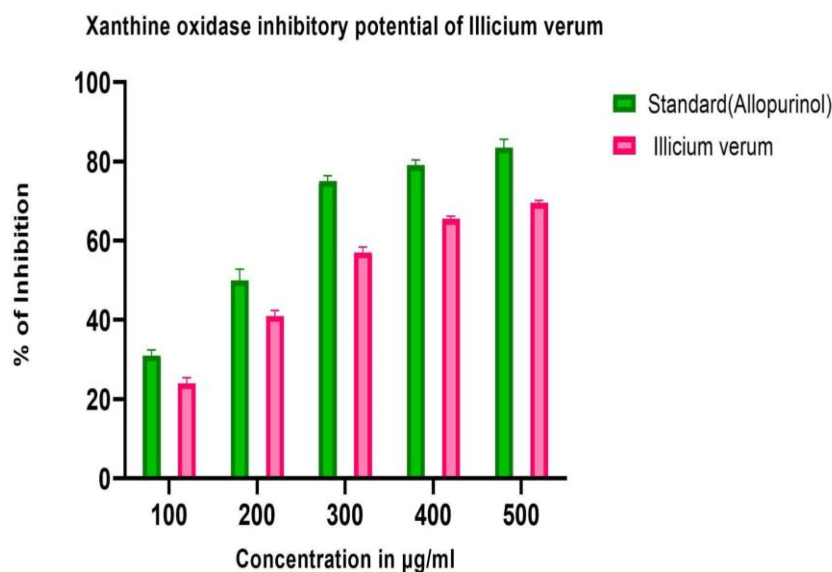

Figure 2. Represents the Xanthine Oxidase Inhibitory Potential of Ethanolic Seed Extract of *Illicium Verum* Compared with the Standard Allopurinol

Discussion

The present study revealed the presence of phytochemicals such as alkaloids, steroids and terpenoids [table1]. Presence of these phytochemicals indicates that the extract can be a potential antioxidant, anticancer and antidiabetic agent. Thus, further research must be done to exhibit medicinal properties of the ethanolic extract of *Illicium verum* (star anise). In the study done by [15], phytochemical analysis of *Illicium verum* was done and presence of phytochemicals such as tri-terpenoids, alkaloids, steroids and flavonoids was proved to be present in *Illicium verum.* In the study done by [16], it was proved that phytochemicals such as alkaloids, steroids, proteins, flavonoids, tannins and phenolic compounds were present. It was also proved that *Illicium verum* has high mineral content.

In the present study, antioxidant properties of ethanolic extract of *Illicium verum* was analysed using DPPH free radical scavenging activity. Free radicals are molecules possessing unpaired electrons emerging from oxidative stress. The effect of antioxidant on DPPH free radical scavenging was due to its hydrogen donating ability. *Illicium verum* being a commonly used spice, is rich in aromatic phytochemical such as alkaloids and thus can scavenge free radicals effectively and act as a potential antioxidant. The result obtained in the study of ethanolic extract of *Illicium verum* has significant antioxidant activity (IC_{50}= 370 microgram/ml). In the study done by [17] antioxidant activity of *Illicium verum* and its protective effect on hydrogen peroxide induced DNA damage of human peripheral lymphocyte cells was proved. In this study antioxidant activity of *Illicium verum* was analysed using lipid peroxide inhibitory activity. In the study done by [18], it was found that essential oil of star anise had lower antioxidant activity than the solvent extract. Thus, in the current study methanolic extract of *Illicium verum* exhibits a significant antioxidant potential.

In the present study it was observed that the standard drug allopurinol exhibited strong anti-gout activity than the extract in all concentrations. Though the synthetic drug is more potent than the herbal extract, it has been reported to exhibit various side effects on a long-time exposure.

Gout is an inflammatory disorder and with synthetic drugs there is no reported cure except for its management. Thus, more research is done to explore herbal extract with anti-gout potential. In a previous study [19], it was proved that hydromethanolic extract of *Erythria stricta* possess xanthine oxidase inhibitory activity. The study done by [20] , also proved that *Eugenia uniflora* possesses xanthine oxidase inhibitory activity due to the presence of flavonoids, queratrin, myricitrin and myricetin.

Thus, the current study also exhibited the anti-gout potential of ethanolic extract of *Illicium verum.* Though possessing less potential than the synthetic drug, further research has to be done to purify the principal component of the herbal extract and prepare a formulation with other identified herbal constituents.

Conclusion

People suffering from gout experience extreme pain in the region of inflammation due to tissue damage. Gout can be treated with medications which inhibit the production of xanthine oxidase. Standard drug for gout (allopurinol) is proven to exhibit a wide range of side effects such as renal dysfunction, hepatic dysfunction, hypersensitivity etc. Thus, synthetic drugs can be replaced with herbal formulations. The present study proved the antioxidant and xanthine oxidase inhibitory activity (antigout) of *Illicium verum.* Further research on the herbal extracts with xanthine oxidase inhibitory potential, especially *in vivo* studies, identification of active principles, pharmacokinetics needs to be elucidated to decrease the potential risk caused due to Gout.

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Conflict of Interest

The authors hereby declare that there is no conflict of interest in this study.

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