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A STUDY ON ANTIOXIDANT, ANTIMICROBIAL AND PHYTOCHEMICAL ANALYSIS OF Illicium verum (STAR ANISE)

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ABSTRACT

People's interest towards herbal medicines has drastically increased in recent years due to its minimum side effects. As our country is abundantly blessed with different medicinal plants, their usage can help us in treating different diseases. As the plants contain various phytocompounds they have the ability to fight against pathogens and also possess antioxidant activity. Thus, these medicinal plants can be used as a promising alternative to treat infections. The current research work focusses on the ability of Illicium verum (Star anise) to act against pathogens. The plant extracts were prepared and analyzed for its phytocompounds. It was also evaluated for its antioxidant and antimicrobial activity. The essential oil prepared from the plant was also evaluated for its antimicrobial activity.

KEYWORDS : antimicrobial, antioxidant, essential oil, Illicium verum, phytocompounds

INTRODUCTION

Nature provides us with abundant wealth. Several natural products obtained from plants are used for different forms of life. They are utilized for its incredible benefits and they have the ability to act against various disease and infections. The phytocompounds present in plants give them the capability to treat different ailments. *Illicium verum*, commonly known as star anise, belongs to family *Illiciaceae*. It has several medicinal values such as improving digestion and stomach related sickness. It was also used in treatment of bronchitis, cough, lung irritation, etc. It was also found to possess anti-parasitic, anti-viral, anti-bacterial and anti-fungal properties. It was traditionally used as an adjunctive therapy for spasmodic colitis pain. *I.verum* fruit extracts were observed to be effective against *A.baumannii*, *Paeruginosa* and *S.aureus*. It was also found to possess anti-fungal activity against *A.niger*, *C.albicans*, *E.fluccosum*, *M.canis* and *T.mentagrophytes*. The fruit was also observed to possess anti-oxidant and anti-inflammatory activity.

MATERIALS AND METHODS:

3.1. Collection Of Samples

The samples of Star anise were collected from the country drug store in Coimbatore district, Tamil Nadu, India. The fruit sample was dried, grounded to a fine powder and used for extraction.

3.2. Extraction Method

About 10 grams of the dried powdered star anise fruits were dissolved in 100 ml of methanol, agitated in an orbital shaker for 2 hours. Whatmann No.1 filter paper was used for filteration. The solvent was evaporated in an oven at 40°c. This extract was then labelled and stored at 5°C for further studies.

3.3. Phytochemical Analysis

3.3.1. Test For Alkaloids

About 1 ml of test solution was taken and 2N HCl was added to it followed by addition of few drops of Mayer's reagent. The white turbidity or precipitate formation indicates the presence of alkaloids.

3.3.2. Test For Amino Acid

About 1 ml of test solution was taken and 1% Ninhydrin in alcohol was added to it. Blue or violet colour formation indicates the presence of amino acids.

3.3.3. Test For Carbohydrate

To about 1ml of test solution, 1ml of 10 % sodium hydroxide was added. The Brown colour production upon heating indicates the presence of carbohydrate.

3.3.4. Test For Flavanoids

About 1 ml of test solution was taken and few drops of 1% ammonia solution was added to it. Yellow colour formation indicates the presence of Flavonoids.

3.3.5. Test For Reducing Sugar

About 5-6 drops of Tollen's reagent was added to 1ml of extract. Appearance of Silver white colour denotes the presence of Sugar.

3.3.6. Test For Terpenoids

About 2 ml of chloroform was added to 5ml of test solution followed by careful addition of 3 ml of concentrated sulphuric acid. Reddish-brown colour formation at the interface confirms the presence of Terpenoids.

3.3.7. Test For Saponins

The test solution and distilled water in the ratio 1:1 was added and mixed well. Foamy lather formation indicates the presence of Saponins.

3.3.8. Test For Steroids

About 1ml of test solution was taken and 3-4 drops of chloroform, few drops of acetic acid, acetic anhydride and 2 drops of concentrated sulphuric acid was added and gently heated. Blue or green colour development confirms the presence of Steroids.

3.3.9. Test For Phenolic Compounds

About 1 drop of ferric chloride was added to 1ml of test solution. Intense colour development indicates the presence of phenolic groups.

3.3.10. Test For Tannins

About 1 ml of test solution was taken and to it few drops of water and lead acetate was added. White precipitate formation indicates the presence of Tannins.

3.4. Antioxidant Assay

DPPH assay method was used with Ascorbic acid as standard. The extract was given for evaluation of antioxidant activity to Bharat Ratna Prof.CNR RAO Research Centre, Coimbatore.

3.4.1. Dpph Scavenging Assay

Different concentrations (1, 2.5, 5 and 10 mg/mL) of methanolic extracts of about 100 μl was added to 5 mL of the DPPH solution (0.005% methanol solution). It was incubated for 30 minutes at room temperature and the absorbance was read against pure methanol at 520 nm. The radical-scavenging activities of the samples were calculated as percentages of inhibition according to the following equation:

$$DPPH\% = \frac{A\,blank - A\,sample}{A\,blank} \times 100$$

where,
A blank - absorbance of the control (containing all reagents except the test compound)
A sample - absorbance of the test compound.

Values of the extracts were compared with those values of BHT.

3.5. Antimicrobial Activity
3.5.1. Test Organisms
The evaluation of antimicrobial activity of the extract was performed against different bacterial strains such as *Bacillus sp, Escherichia coli, Klebsiella sp, Pseudomonas sp, Proteus sp, and Staphylococcus aureus*. The isolates were maintained in nutrient agar and sub-cultured regularly. The culture was incubated at 35-37°C for 24 hours.

3.5.2. Well Diffusion Bioassay
About 0.1ml of each bacterial strain was spread on sterilized nutrient agar plates with sterile swabs. A sterile cork borer was used to punch the agar medium to a size of 6mm and different concentrations of the samples such as 50 μl, 100 μl, 150 μl, 200 μl was added with a micropipette. The plates were allowed to stand at room temperature for 30min and incubated at 37°C for 24 hours. The zone of inhibition was measured (mm) after incubation.

3.6. Oil Extraction
The powdered form of *I.verum* was mixed with coconut oil and was left undisturbed for about two days. Later the oil was filtered and used for further analysis.

3.7. Antimicrobial Activity For Oil Extract
3.7.1. Well Diffusion Bioassay
About 0.1ml of each bacterial strain was spread on sterilized nutrient agar plates with sterile swabs. A sterile cork borer was used to punch the agar medium to a size of 6mm and different concentrations of extracted oil samples of about 50 μl, 100 μl, 150 μl, 200 μl was added with a micropipette. The plates were allowed to stand at room temperature for 30min and incubated at 37°C for 24 hours. The zone of inhibition was measured (mm) after incubation.

RESULT AND DISCUSSION:
4.1. Phytochemical Analysis
Phytochemical constituents of *Illicium verum* was analyzed and tabulated (Table no. 1)

Table 1
Phytochemical Analysis Of *I.verum*

PHYTOCHEMICAL TEST	RESULTS
Alkaloids	+
Amino acids and proteins	+
Carbohydrate	+
Flavonoids	+
Phenol	+
Reducing agents	+
Saponins	+
Tannis	+
Trepenoids	+
Steroids	+

(+) indicates Positive; (-) indicates Negative

The plant extract was found to contain various phytochemicals such as aminoacids, proteins, flavonoids, phenolic compounds, carbohydrates, tannins, etc which provide them several properties such as antioxidant, antimicrobial etc.

4.2. Antioxidant Assay
The fruit extract of *I.verum* was found to be effective in exhibiting antioxidant activity against hydrogen peroxide and also offered protection against DNA damage. Researchers found that high content of polyphenols was responsible for the potential antioxidant activity.

Table 2:Antioxidant Assay For *I.verum*

CONCENTRATION (ml)	% OF INHIBITION
50	89.57
100	96.52
150	95.65
250	93.04
350	90.43
750	77.39

4.3 Antimicrobial Activity Of Methanol Extract
Table 3:Antimicrobial Activity Of *I.verum* Extract

S.NO	ORGANISMS	ZONE OF INHIBITION (mm)			
		50 μl	100 μl	150 μl	200 μl
1	Klebsiella sp	11	12	14	20
2	Bacillus sp	10	11	13	35
3	Proteus sp	11	13	14	19
4	Pseudomonas sp	10	11	13	15
5	S.aureus	10	11	12	23
6	E.coli	10	11	14	22

The well diffusion assay showed that the plant extracts have different degrees of bacterial growth inhibition, depending on the strains. Methanol extract of *Illicium verum* showed better antimicrobial activities against medically important bacterial strains. It was found to be more effective against *Klebsiella sp* and *S.aureus*.

4.4 Antimicrobial Activity Of Oil Extract Of Sample
Table 4:Antimicrobial Activity Of *I.verum* Oil Extract

S.NO	ORGANISMS	ZONE OF INHIBTION (mm)			
		50 μl	100 μl	150 μl	200 μl
1	Klebsiella sp	16	19	20	24
2	Bacillus sp	12	16	22	24
3	Proteus sp	-	15	16	-
4	Pseudomonas sp	-	15	22	-
5	S.aureus	18	19	21	24
6	E.coli	14	17	20	26

CONCLUSION:
In this study *Illicium verum* (Star anise) was collected, grounded into fine powder and extracted by using methanol. The extract was then used for further studies. The phytochemical analysis showed different phytocompounds present which are responsible for various activities of the plant. The bioactive components include alkaloids, flavonoids, phenols, tannins, proteins, etc. The extract also showed good radical scavenging activity. The antioxidant activity was found to be increasing with increase in concentration. Methanolic extract of *Ilicium verum* also showed better antimicrobial activity against different isolates. The essential oil extracted from the plant has also showed effective activity against pathogens.

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