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In vitro antibacterial and antifungal potential of *Illicium verum*

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ABSTRACT

This study investigated the *in vitro* antibacterial and antifungal activity of different solvent extracts of *Illicium verum* fruit. The aqueous, acetone, ethanol, hexane, petroleum ether, chloroform extract showed significant results against five gram-positive bacteria (*Micrococcus luteus, Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Streptococcus pyogenes*), five gram-negative bacteria (*Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Salmonella paratyphi, Enterobacter aerogenes*) and five fungi (*Aspergillus niger, Aspergillus flavus, Candida albicans, Alteria alternata, Candida kefyr*) by disc diffusion method. The susceptibility of the test microbes varied with the type of solvent used. Among the all solvents, the polar solvents showed better antibacterial and antifungal potentiality than the non-polar solvents. This observation indicates that the presence of polar solvent soluble bioactive compounds responsible for efficient antimicrobial activity *I.verum*. Thus *I.verum* can be used as ethanomedicine to treat the infectious diseases caused by these pathogens.

Keywords: *Illicium verum*, Fruit extract, Antibacterial, Antifungal.

INTRODUCTION

Nature has bestowed on us a very rich botanical wealth. Traditional systems of medicine, modern medicines, neutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs are directly or indirectly dependent on plants[1]. Actually the medicinal value of plants lies in some chemical substances that produce a definite physiologic action on the human body[2]. Recently WHO introduced guidelines on research and evaluation of traditional medicine and practice. This guideline has a major objective of developing traditional medicine leads into standardized and scientifically validated drugs, it aims to ensure quality and safety of botanicals before being evaluated for its efficacy[3]. The screening and evaluation of medicinal plants is very dependent on the proper cultivation and collection of the plant materials followed by their extraction and isolation of the phytochemical entities to enable optimized bioactive compound production and subsequent therapeutic applications. This is very important for multi-component drugs and their standardized extracts to ensure high quality and batch-to-batch consistency [4].

Infectious diseases are the leading causes for death. Nowadays, multiple drug resistance has been developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of the infectious diseases. In addition, antibiotics are sometime associated with adverse effects on the host including hypersensitivity, immuno suppressant and allergic reactions. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants[5]. Antimicrobial agents of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials[6]. The present study was aimed to elucidate the antibacterial and antifungal properties of various solvent extracts of *Illicium verum* against different pathogens.

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MATERIALS AND METHODS

Collection of plant material

Disease free, healthy fruits were collected from Chennai, India. The seeds were separated from the fruit and shade dried for ten days at room temperature. Then the dried fruits were grinded to fine powder and stored in sterile airtight container in refrigerator.

Preparation of crude extract

Ten grams (10g) of the ground plant samples were separately soaked in 200 ml of each solvent and allowed to stand for about 72 h for extraction. After the 72 h, it was then filtered using Whatmann No. 1 filter paper. The filtered samples were sterilized by passing through millipore filter and later evaporated to dryness[7].

Test microorganisms

To evaluate the antibacterial and antifungal potential the following test microorganisms, gram-positive bacteria including *Micrococcus luteus, Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Streptococcus pyogenes*, gram-negative bacteria including *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Salmo-*

nella paratyphi, Enterobacter aerogenes, fungal strains including *Aspergillus niger, Aspergillus flavus, Candida albicans, Alteria alternata, Candida kefyr* were used and maintained on Mueller Hinton agar (MHA- Hi Media, Mumbai, India) (for bacteria) and potato dextrose agar (PDA- Hi Media, Mumbai, India)) (for fungi) at 4°C.

Inoculums preparation

Bacterial inoculums were prepared by growing cells in Mueller Hinton Broth (MHB) (Hi Media, Mumbai, India) for 24 h at 37 °C. These cell suspensions were diluted with sterile MHB to provide cell counts of about 10^8 CFU/ml and used for antibacterial study. For fungal inoculums, the fungal strains were grown on PDA for 48h at 37 °C. Then the spores were suspended in double distilled water and homogenized and used for fungicidal study.

Antibacterial assay

Antibacterial activity of different solvent extract was evaluated by agar disc diffusion method on MHA plate[8]. The bacterial suspension was spread on solid plate using sterile swab aseptically and allowed to dry. The discs loaded with extract residues were placed on top of the seeded medium and gently pressed to ensure contact. Plates were kept at room temperature for 30 minutes as pre-diffusion time. Ampicillin (10µg/disc) and blank discs impregnated with the respective solvents were used as positive control and negative control respectively. The plates were incubated for 24 h at 37°C. Inhibition zones formed on the medium were evaluated in millimeter and the individual test was performed for three times.

Antifungal assay

The antifungal activity of the extract was tested by disc diffusion method[9, 10] on PDA plate. The solid plate was moistened by fungal suspension with sterile swab and allowed to dry. The plant extract disc was plated aseptically on the seeded plate and confirmed the attachment. The plates were kept at room temperature for 30 min for compound diffusion. Blank disc impregnated with each solvent followed by drying off was used as negative control and standard antifungal disc flucanozole (10µg/disc) was used as positive control. The activity was determined after 72 h of incubation at 37°C. The diameter of zone of inhibition produced by the extract was measured in millimeter. Each sample was used in triplicate for the determination of antifungal activity.

RESULTS

The antibacterial and antifungal efficiency of fruit extract of *Illicium verum* was evaluated *in vitro* against five gram-positive, five gram-negative and five fungal strains. The extract was obtained using three polar solvents such as acetone, ethanol, aqueous and three non-polar solvents like hexane, petroleum ether, and chloroform. All the extracts showed significant potentiality against these isolates. Among these, fruit extract showed better results against the bacterial isolates (Table 1) that the fungal isolates (Table 2). The presence of broad spectrum of antibacterial and antifungal activity of *I. verum* implies that it can be used as good drug candidates.

Antibacterial activity

In the current study, ethanol extract was showed maximum zone of inhibition against gram-positive bacteria among the polar solvents (Table 1). This extract was found to be more effective against *Streptococcus pyogenes* (22± 0.18) followed by *Staphylococcus aureus* and *Bacillus cereus* with zone of inhibition (21± 0.68 and 21±0.18) respectively. *Micrococcus luteus* and *Bacillus subtilis* were moderately inhibited by ethanol extract. Aqueous extract also showed significant result against *Bacillus subtilis* (21±0.32) among the all gram-positive bacteria, even this extract was effective than the positive control one. The ethanol extract also produced better result against gram-negative bacteria. The maximum zone of inhibition was produced for *Pseudomonas aeruginosa* (19± 0.12), followed by *Klebsiella pneumoniae* (18± 0.12). Similarly for gram-negative bacteria, after ethanol extract, aqueous extract showed better result against them. Highest zone of inhibition was produced for *Escherichia coli* (18±0.00), then for *Pseudomonas aeruginosa* (15± 0.09) by the water extract of *I. verum*. Among the non-polar solvents, chloroform extract followed by hexane extract exhibited efficient result, but petroleum ether was not showed the significant results. Among the gram-positive bacteria, the maximum zone of inhibition was observed against *Staphylococcus aureus* (13±0.07) by chloroform extract, *Bacillus subtilis* (11±0.57) by hexane extract *Micrococcus luteus* (10±0.19) by petroleum extract whereas in case of gram-negative bacteria, chloroform extract showed same result for *Pseudomonas aeruginosa* and *Escherichia coli* (10±0.56, 10±0.33) respectively, but hexane extract found to be more effective against *Enterobacter aerogenes*(12±0.72). In this case also petroleum ether did not showed potential inhibition effect.

Table 1. Antibacterial activity of *Illicium verum* fruit extract

Bacterial organisms	Zone of Inhibition (mm)						
	Aqueous	Acetone	Ethanol	Hexane	Pet. Ether	Chloroform	Control
Micrococcus luteus	14±0.21	18± 0.11	20±0.78	8±0.17	10±0.19	11±0.12	17±0.22
Staphylococcus aureus	18±0.01	16± 0.56	21±0.68	9±0.80	8±0.43	13±0.07	18±0.10
Bacillus subtilis	21±0.32	18± 0.45	20±0.43	11±0.54	9±0.01	10±0.12	26±0.15
Bacillus cereus	16±0.87	19± 0.32	21±0.08	7±0.10	8±0.33	11±0.13	24±0.17
Streptococcus pyogenes	11±0.15	15± 0.09	22±0.18	10±0.21	7±0.43	12±0.98	28±0.12
Pseudomonas aeruginosa	15±0.09	15± 0.16	19±0.12	9±0.00	8±0.89	10±0.56	23±0.21
Escherichia coli	18±0.00	16± 0.57	17±0.38	7±0.23	7±0.02	10±0.33	25±0.23
Klebsiella pneumoniae	12±0.03	12± 0.23	18±0.12	7±0.22	7±0.16	9±0.24	23±0.02
Salmonella paratyphi	12±0.33	10± 0.00	15±0.09	8±0.19	9±0.25	8±0.29	15±0.01
Enterobacter aerogenes	10±0.23	11± 0.54	15±0.25	12±0.72	10±0.00	7±0.67	24±0.11

Values are expressed as Mean ± Standard Error of three replicates.

Antifungal activity

Antifungal activity of *I. verum* extract was assayed and the inhibition effect of different solvent extract on the growth of various fungi was showed in Table 2. The data revealed that polar solvents, specialy ethanol and aqueous extract were able to inhibit the growth of maximum fungi isolates. Ethanol extract was found to be effective against *Aspergillus flavus* with highest zone of inhibition (18±0.15) whereas aqueous extract showed potential result against *Aspergillus niger* (18±0.15). But all the non-polar solvents were not efficient like polar solvents. Among the non-polar solvents, chloroform extract, followed by hexane and then petroleum ether produced fungi growth inhibition which is similar results with bactericidal effect. Chloroform and hexane extract produced highest growth inhibition against *Aspergillus flavus* (13±0.07, 14±0.12) respectively.

Table 2. Antifungal activity of *Illicium verum* fruit extract

Fungal organisms	Zone of Inhibition (mm)						
	Aqueous	Acetone	Ethanol	Hexane	Pet. Ether	Chloroform	Control
Aspergillus niger	18±0.15	14± 0.88	16±0.17	11±0.11	9±0.17	10±0.13	21±0.02
Aspergillus flavus	15±0.18	13± 0.32	18±0.15	14±0.12	9±0.13	13±0.07	18±0.12
Candida albicans	15±0.15	12± 0.03	16±0.33	9±0.23	8±0.04	11±0.10	14±0.01
Alteria alternate	13±0.33	13± 0.15	12±0.09	8±0.10	8±0.16	10±0.13	12±0.15
Candida kefyr	10±0.23	11± 0.27	10±0.21	7±0.02	7±0.05	8±0.18	10±0.03

Values are expressed as Mean ± Standard Error of three replicates.

DISCUSSION

There is an ever-increasing demand for plant-based therapeutics in both developing and developed countries due to a growing recognition that they are natural products, non-narcotic and, in most cases, easily available at affordable prices as well as they have no side effects5. Plants are reservoir of many known and unknown chemical compounds, some of these compounds are capable of restricting or retarding the growth of microorganism. Many plants are known to harbor active principle that confers the plants the ability to withstand microbial attack. These plants can be source for compounds that have pharmaceutical implication[11].

The antibacterial and antifungal activities of *Illicium verum* was tested against various gram-positive, gram-negative and fungal isolates. Different extracts of *I. verum* showed significant bactericidal and fungicidal effect. Among the all polar and non-polar solvent, ethanol extract have been found to be more potent inhibitory followed by aqueous, acetone, chloroform, hexane with moderate inhibition and petroleum ether exhibited minimum activity. Different solvents have various degrees of solubility for different phytoconstituents[12]. The antimicrobial activity of medicinal plants and drugs varies in their inhibitory effect, depending on the concentration of crude extracts or synthetic drug, size of inoculums, temperature, nature of organism, and rate of diffusion[13].

Antimicrobial activity of fruit extract might be related to their phenolic compounds. Phenolic compounds are known to be synthesized by plants in response to microbial infection. It is therefore possible that they can act as effective antimicrobial substances against a wide array of microorganisms. However, the antimicrobial activity of plant extracts depends not only on phenolic compounds but also by the presence of different secondary metabolite like hydroxyl groups on the active constituents, because of the ability of these substances to bind to bacterial adhesion and disturb the availability of receptors on surface[14, 15].

Here the activity of the plant was investigated by using three polar solvents namely, aqueous, acetonic and ethanolic and three non-polar solvents, such as, hexane, petroleum ether and chloroform. But maximum zone of inhibition was observed in case of polar solvents than the non-polar solvents. As a whole, all the extract produced better result against gram-positive bacteria compared to gram-negative bacteria. This may be attributed to the fact that these two groups differ in their structure of the cell wall components. The ability of tannin compounds to cause the bacterial colonies to disintegrate, probably results from their interference with the bacterial cell wall[16]. Majority of plant extracts have been re-ported to be more active against gram-positive bacteria than the gram-negative bacteria strains[17, 18, 19]. All the extract has the potentiality on the inhibition of different microbial growth. The extracts were effective against *Micrococcus luteus, Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Streptococcus pyogenes, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Salmonella paratyphi, Enterobacter aerogenes, Aspergillus niger, Aspergillus flavus, Candida albicans, Alteria alternata, Candida kefyr* which are responsible various infectious human diseases. Thus *I. verum* extract can be used as the richest source for wide range of antimicrobial substances.

CONCLUSION

The results indicated that the polar solvent extract of *Illicium verum* showed significant impact against these test microorganisms than the organic solvent. Among the polar solvents, the ethanol solvent exhibited maximum effect. Thus the ethanol extract of *I.verum* confirm the presence of broad spectrum of novel bioactive compounds and can be used to carry out further pharmacological evaluation to be used as antimicrobial drugs.

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