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Preservative Efficacy of *Illicium verum* Hook in Fruit Juices

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ABSTRACT

Fruit juices contain all essential nutrients that help in maintaining the health of human beings. However, fruit juices are easily spoiled by the growth of microorganism. Hence, the current investigation has been focused to examine the preservative potential of *Illicium verum* against microorganisms isolated from juices. Organic and aqueous extracts of *I. verum* fruits were accessed for their antimicrobial efficacy and minimum inhibitory concentration (MIC) against selected microbes. Among the different solvents, acetonic extract was observed to be the most excellent solvent extract and the least MIC was 0.39 mg/ml. Hence, acetonic extract of *I. verum* has a biopreservative efficacy.

Keywords: *Illicium verum*; preservative; antimicrobial; minimum inhibitory concentration

1. INTRODUCTION

The evolution in the food sector technologies is still lacking in preservation of food. The main concern of the food authorities as well as the food industry is the safety of food[1]. Freshness and naturalness of the food draws the attention of the consumers towards the food. Fruit juices comprise all the essential nutrients such as antioxidants, vitamins and minerals that assist in the circumventing the health risk problems in human[2-3]. However, they also provide the good nutrients for the growth of the microorganisms. The most commonly encountered genera in fruit juices are acid tolerant bacteria (*Acetobacter sp, Alicyclobacillus sp, Bacillus sp, Gluconobacter sp, Lactobacillus, Leuconostoc sp, Zymomonas, Zymobacter, Propionibacterium* and members of Enterobacteriaceae (*Klebsiella*., *Citrobacter* sp. and *Serratia* sp.) together with yeasts (*Pichia, Candida, Saccharomyces* and *Rhodotorula*[4] and moulds[3,5]. *Escherichia coli* and different serovars of *Salmonella sp* implicated in several food borne outbreaks link with the consumption of unpasteurised fruit juices[2,3,6].

To curb the growth of microorganisms in foods, several technologies including thermal and non-thermal processing have been employed in foods. However, pasteurization leads to loss of some bioactive compounds in fruit juices. Pulsed electric field (PEF) is an emerging non-thermal technique but high intensity and longer period utilised in PEF affect the nutritionary quality of foods[2,7].

Sodium benzoate and potassium sorbate (chemical preservatives) are commercially applicable in the permissible level of 0.1 % in USA and 0.15 % - 0.25 % in several other countries[8]. Literature cited the harmful effects of chemical preservatives in foods lay down the pressure to control the use of chemical preservative[9-10]. Therefore, the attention has been diverted towards the use of natural occurring antimicrobial compounds from plants in food preservation. The purpose of utility of spices and herb in food/medicines to maintain proper cleanliness conditions in India[8].

llicium verum Hook.f. is an aromatic evergreen tree widely distributed in southern China alongwith Vietnam and India. It is commonly known as Chinese star anise and Badyan, belongs to *Illiciaceae* family[11-12]. It consists of purple-red flowers, star-shaped anise-scented fruits as shown in Fig. 1[1]. It acquires the GRAS regulatory status (GRAS 21 CFR 182.10 CFR- Title 21).

Figure 1. *Illicium verum* fruits.

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The crude extract and essential oil of *I. verum* possess several pharmacological properties such as antimicrobial, antioxidant and insecticidal[11,13]. It is the important resource of shikimic acid which is the chief component in anti-influenza virus drug Tamiflu[14]. The pharmacological attributes of *I. verum* is owing to the presence of active metabolites like phenypropanoids, flavanoids and terpenes[11]. Trans-Anethole is the major compound observed in *I. verum* extracts imparting its antimicrobial properties[15].

The core point of the present examination was to decide the *in vitro* antimicrobial action of *Illicium verum* fruits in various solvents against the microorganisms related with fruit juices. The structural elucidation of the compound through GC – MS analysis established the relationship between antimicrobial activity and chemical compounds.

2. MATERIALS AND METHODS

2.1 Plant Collection

The fruits of *I. verum* were procured from the local market in Yamunanagar and its taxonomic identity was proved by key provided in Wang[11], *et al*.

2.2 Extraction of Plant Material

The procedure for the extraction of plant material was adopted from previous study[4,12,16.]

2.3 Test Microorganisms

Microorganisms were selected and identified[17] on the basis of previous study[3] .

2.4 Screening for Antimicrobial Activity Against Microorganisms

The antimicrobial efficacy of *I. verum* fruits in different solvents were accessed by the agar well diffusion method against bacteria and yeast and by poison food technique against moulds[18-19].

2.5 Minimum Inhibitory Concentration Determination

Minimum inhibitory concentration was tested by by the broth macrodilution method[9,16].

2.6 Statistical Analysis

The obtained results were statistically assessed utilising SPSS programming 16 at 5 per cent significant level. Means were looked at utilising Tukey's simultaneous test set at P <0.05.

2.7 GC-MS Analysis of Acetonic Extract

GC-MS analysis of the acetonic extract of *I. verum* fruit was access through Advanced Instrumentation Research Facility, Punjab University, Chandigarh.

3. RESULTS AND DISCUSSION

3.1 Effect of the Solvent on the Antimicrobial Activity

In India, spices are not only used in food as flavoring agent but also as medicines in the treatment of several ailments. Spices comprise several active metabolites that confer its antimicrobial properties. The contrast of plant antimicrobial properties are grueling task owing to the employment of different method of extraction, solvent, and the tested microorganism[4,18,21-23].

In the present study, the antimicrobial efficacy of *I. verum* fruits extracts differed in several organic and aqueous extracts. Positive control displayed 15.6 mm - 20 mm inhibition zones against the tested bacteria and 14.6 mm against the yeast, whereas negative control did not show activity. The data in Table 1 explained that all solvent extracts comprised antimicrobial effect against the tested bacteria *and R. mucilaginosa,* however aqueous extracts displayed not antimicrobial activity against *R. mucilaginosa*. Among all the plant extracts, acetonic extract was discovered best against *B. cereus*. The obtained results established that organic extracts of fruits exhibited better antimicrobial activity than sodium benzoate.

Table 1. Antimicrobial potential of *I. verum* fruits

Solvent extracts (mg/ml)	Antimicrobial activity of different extracts (mm)		
	B. cereus	***S. marcescens***	***R. mucilaginosa***
Acetone	34.3_{bx}±1.52	27.3_{by}±0.57	25.3_{bz}±0.57
Methanol	29.3_{cx}±0.57	23.3_{cy}±0.57	22.3_{cz}±0.57
Ethanol	25.3_{dx}±0.57	20.6_{dy}±0.57	19.3_{dz}±0.57
Cold aq	19.6_{ex}±0.57	14.3_{ey}±0.57	-
Hot aq	13.6_{fx}±0.57	12.3_{fy}±0.57	-
Control	20±1.56	15.6±0.57	14.6±0.57
DMSO	-	-	-

(-) = no activity,

The percentage mycelial inhibition of solvent extracts (organic and aqueous) varied from 14.3 mm - 40.6 mm for *A. flavus* and for *P. citrinum* varied from 12.6 mm - 38.6 mm. Positive control displayed significant mycelial inhibition in the range of 26.5 mm - 30.8 mm. The similar trend was observed in amtimould activity of plant extracts. Acetonic extract of *I. verum* revealed the best antimould efficacy against *A. flavus* (Percentage mycelia growth inhibition = 40.6 mm) followed by methanolic extract (38.3 mm) and same results had been observed for *P. citrinum* as shown in Table 2.

The MIC values ranging from 0.39 mg/ml - 100 mg/ml was observed against different fruit juice associated

Table 2. Antimicrobial efficacy of *I. verum* fruits against moulds

Solvent extracts mg/ml	Percent mycelial growth inhibition	
	A. flavus	***P. citrinum***
Acetone	40.6_{bu}±0.57**	38.6_{bv}±0.57
Methanol	38.3_{cu}±0.57	35.6_{cv}±0.57
Ethanol	28.6_{du}±0.57	27.3_{dv}±0.57
Cold aq	15.6_{eu}±0.57	13.3_{eu}±0.57
Hot aq	14.3_{fv}±0.57	12.6_{fv}±0.57
Control	30.8±0.57	26.5±0.57
DMSO	-	-

*- Values, percentage mycelial inhibition means of duplicates; (-) no activity

Table 3. MIC of *I. verum* fruits extracts

Solvent extracts (mg/ml)	MIC of different extracts (mg/ml)				
	B. cereus	*S. marcescens*	*R. mucilaginosa*	*A. flavus*	*P. citrinum*
Acetone	0.39	0.78	3.12	3.12	3.12
Methanol	0.78	6.25	6.25	3.12	3.12
Ethanol	3.12	12.5	25	6.25	6.25
Cold aq	12.5	50	Nt	50	50
Hot aq	50	Nt	Nt	100	Nt

Nt- not tested

microorganisms in the present investigation. Acetonic extract of fruit showed the lowest MIC of 0.39 mg/ml was observed against *B. cereus* as shown in Table 3.

The organic extracts displayed more activity in contrast to aqueous extracts in the present study. Similar trend has been recorded in several studies of plant extracts[4,16,18]. Only few studies are available in literature related to antimicrobial potential of *I. verum*. The antimicrobial efficacy of methanol, ethyl acetate and hexane extract of *Illicium griffithii* Hook. f. was studied against *Staphylococcus aureus*, *Yersinia enterocolitica*, *Vibrio parahaemolyticus* and several clinical isolates by disc diffusion method in the range of concentration 1.25 mg/disc to 5 mg/disc. Among these solvent ethyl actetae extract of *I. griffithii* fruit was most effective against these tested microbes. Myristicin (29.20 %) and linalool was the major compound encountered in the ethyl acetate and hexane extract of *I. griffithii* fruits[24]. In another study, essential oil of *I. verum* was also blended in the packaging material to study its potential in increasing the shelf life of the food. Essential oil *I. verum* blended in ß-Cyclodextrin-was effective in the reduction the growth of *Penicillium expansum* in apple[25].

Water ethanol (20:80) extract of *I. verum* possessed the anti quoram sensing and anti biofilm formation in milk against *Staphylococcus aureus*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, and biosensor strain *Chromobacterium violaceum*[14]. Essential oil, metanolic and ethanolic extract of *I. verum* fruit showed the mycelial growth inhibition against *Aspergillus flavus*, *Aspergillus parasiticus* and *Fusarium verticillioides*[13].

3.2 Sensitivity of the Tested Microorganisms

The tested microorganism were displayed varying sensitivities to the 5 tested extracts as shown in Tables 1 and 2. On the basis of MIC and diameter of inhibition zone, *B. cereus* was more vulnerable microorganism and the most hardest one was *R. mucilaginosa*. This finding was persistent with the work of the earlier study[12-13]. De[12], *et al.* studied the antimicrobial activity of star anise and isolated pure compound anethole. The MIC value of extract of *I. verum* was in the range of 5 µg/ml-10 µg/ml against *B. subtilis*, *B. licheniformis* , *B. megaterium* and *B. cereus* while MIC in the range of 10-50 µg/ml against several species of gram negative bacteria. The value of MIC was higher in case of gram negative bacteria than gram positive bacteria. The same outcome was observed in present study. Value of MIC was 30 µg/ml ans 20 µg/ml for *A. flavus* and *Penillium chrysogenum,* respectively. The difference in the sensitivity between the gram positive and gram negative bacteria is based on the difference in their cell wall composition. The essential oil and other secondary metabolites easily penetrate into the cell wall of gram positive bacteria in comparison to gram negative bacteria that contain more lipid content[6,18,21].

3.3 GC-MS analysis of *I. verum* fruit extract

The crude acetonic extract of *I. verum* fruits was studied for their chemical composition using GC-MS analysis. Area normalization was used to count the relative quantity of each component. A total of 11 compounds were identified (Table 4) on the 11 peaks (Fig. 2) comprising of mainly Diisooctylphthalate (26.68 %), Bis (2ethylhexyl) phthalate (28.01 %), 9Octadecenamide (8.34 %), .

Peng[27], *et al.* reported the compounds present in the *I. verum* fruit extract obtained by four stage extractants (YBSJ extract) by the GC-MS analysis. The key components of *I. verum* fruit in this study was acetic acid, anethole, benzaldehyde and many more. Essential oil of *I. verum* contain trans-Anethole as main compound[15]. Myristicin and linalool were the major

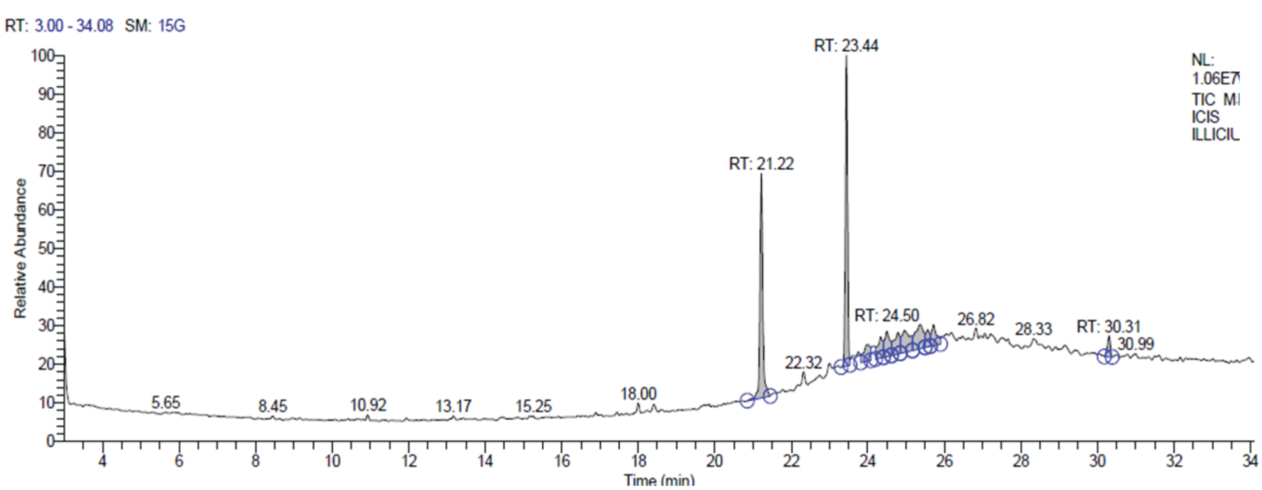

Figure 2. Ion chromatogram of *I. verum* fruit acetonic extract.

Table 4. GC –MS analysis data of *I. verum* acetone crude extract

Retention time	Compound name	Molecular formula	Area (%)	Cas#
21.22	Diisooctylphthalate	$C_{24}H_{38}O_4$	26.68	131204
23.44	Bis(2ethylhexyl)phthalate	$C_{24}H_{38}O_4$	28.01	117817
23.99	Rhodopin	$C_{40}H_{58}O$	4.58	105920
24.33	1Monolinoleoylglyceroltrimethylsilylether	$C_{27}H_{54}O_4Si_2$	4.37	54284456
24.50	9,12,15Octadecatrienoicacid,2,3bis[(trimethylsilyl)oxy]propylester,(Z,Z,Z)	$C_{27}H_{52}O_4Si_2$	5.37	55521227
24.79	2[4methyl6(2,6,6trimethylcyclohex1enyl)hexa 1,3,5trienyl]cyclohex1en1carboxaldehyde	$C_{23}H_{32}O$	5.32	NA
24.95	.psi.,.psi.Carotene	$C_{42}H_{64}O_2$	7.56	13833017
25.36	9Octadecenamide	$C_{18}H_{35}NO$	8.34	3322621
25.56	3,4]benz[1,2e]azulene4a,5,7b,9,9 a(1aH)pentol	$C_{28}H_{38}O_{10}$	2.71	77698374
25.72	Octasiloxane	$C_{16}H_{50}O_7Si_8$	4.48	19095240
30.31	3Desoxo3,16dihydroxy12desoxyphorbol 3,13,16,20tetraacetate	$C_{28}H_{38}O_{10}$	2.58	NA

compounds reported in ethyl acetate and hexane extract of *I. griffithii* fruit[24]. Limited data is available about the preservative potential of *I. verum* fruit extract. The presence of phthalates in the acetonic extract might be responsible for the antimicrobial activity of the compounds.

4. CONCLUSIONS

I. verum is versatile plant that exhibit several pharmacological and medicinal properties. However, there is limited data available in literature for the preservative action of *I. verum* in foods. The outcome of the present investigation ascertained that all the extracts of *I. verum* fruits possessed antimicrobial activity against selected microbes associated with juices. Acetonic extract of *I. verum* fruit displayed better antimicrobial activity than commonly used chemical preservative *in vitro* as well as *in vivo*. However, further research work is required to check the permissible level of *I. verum* in juices that can inhibit the growth of microorganisms without affecting the sensory properties of fruit juices.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of paper.

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In the present study, he has contributed in editing as well as updating of the manuscript.