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Phytochemical analysis and *in vitro* antimicrobial activity of *Illicium griffithii* Hook. f. & Thoms extracts

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ABSTRACT

Objective: To evaluate the antimicrobial activity of hexane, ethyl acetate and methanol extracts of seeds and fruits of *Illicium griffithii* (*I. griffithii*) (Family: Schisandraceae). **Methods:** The antimicrobial activity of the organic extracts were determined using disc diffusion assay against Gram-positive bacterial strains (three reference cultures and three clinical isolates), Gram-negative bacterial strains (nine reference cultures and six clinical isolates), and six fungi. The primary phytochemical and chemical compositions were analyzed using qualitative chemical analysis and gas chromatography-mass spectrometry respectively. **Results:** Ethyl acetate extract of fruits was effective against most of the tested reference cultures such as *Staphylococcus aureus*, *Yersinia enterocolitica*, *Vibrio parahaemolyticus*, *Bacillus subtilis*, *Salmonella paratyphi*, *Enterococcus feacalis*, *Xanthomonas oryzae* and *Pseudomonas aerugenosa*, whereas methanol extract showed activity only against *Staphylococcus aureus*, *Bacillus subtilis and Xanthomonas oryzae*. The hexane and ethyl acetate extracts of fruits were more effective against most of the clinical isolates, whereas methanol extract was effective only against *Klebsiella pneumoniae* ESBL. The extracts of fruits and seeds did not show any significant antifungal activity against tested fungi. The presence of phenols, tannins, flavonoids, triterpenoids, steroids, alkaloids, saponis and carbohydrates in the different extracts was established. Gas chromatography-mass spectrometry studies on hexane and ethyl acetate extract of fruits resulted in the identification of 31 and 39 compounds respectively. **Conclusions:** Potent antibacterial phytochemicals are present in ethyl acetate extract of *I. griffithii* fruits. Further studies are needed to investigate activities of *I. griffithii* against multidrug resistant bacteria.

1. Introduction

Despite of tremendous progress in human medicines, infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health[1]. Nature has provided an important source of remedies to cure all the ailments of mankind. In recent years, all the medicines used were from natural source, especially from plants[2]. Plants contain hundreds or thousands of metabolites. Medicinal and aromatic plants, a gift of nature, are being used against various infections and diseases in the world since past history. Only a small percentage of plants species have been investigated phytochemically and the fraction submitted to biological screening is even smaller[3]. Plant kingdom represents an extraordinary reservoir of novel molecules. Plant derived products have been used for medicinal purposes for centuries[4] and plants have been an important source of medicine for thousands of years[5]. Many plants are used as folk medicines to infectious diseases such as urinary tract infections, diarrhea, coetaneous abscesses, bronchitis and parasitic diseases[6].

Illicium is the sole genus in the family Illiciaceae. It comprises of 42 species of evergreen shrubs and small trees. The species are native to the tropical and subtropical regions of eastern and southeastern Asia, southeastern North America and the West Indies[7]. The most frequently occurring species are *Illicium dunnianum* (*I. dunnianum*) and *Illicium griffithii* (*I. griffithii*)[8], *Illicium verum* (*I. verum*) and *Illicium anisatum* (*I. anisatum*). *I. griffithii* Hook. f. & Thoms is one of the most frequently occurring *Illicium* species in the world. In Arunachal Pradesh, it

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is locally known as "Lissi". It is a small to medium sized tree, which occurs in subtropical and temperate broad-leaved forests of West Kameng, Tawang, Lohit and Lower Subansiri districts and is widely distributed in Bomdila, West Kameng district. It possesses potent antimicrobial activity[9]. *I. griffithii* is an important medicinal tree species of the temperate broad-leaved forests of Northeast India[10]. Fruits of *I. griffithii* are used in the pharmaceutical and spice industries[11]. In recent years, scientists also found cancer fighting properties especially against lung cancer cells. These reports are sufficient to highlight the need of this species in near future as well[12].

Traditionally, dried seedless fruit is used as incense. It is used for sweet fragrance while preparing butter-salted tea or sugar tea. They are also used as medicine to cure cough, toothache and sinusitis (by inhaling the vapour or by boiling the fruits in water) and to improve the strength of local alcohol[13]. The fruit is considered to be carminative, stomachic and glactagogic. It is also used in treating vomiting, dyspepsia, abdominal pain and food poisoning[14]. The key active component involved in the synthesis of Tamiflu is shikimic acid[15], which can only be effectively isolated from Chinese star anise (*I. verum*)[16]. There is a growing demand for star anise as a source of shikimic acid for the manufacture of anti-viral drugs widely used in the treatment and prophylaxis of avian flu (commonly bird flu). Shikimic acid is the starting compound utilized for the manufacture of the anti-viral drug oseltamivir[17]. The main objective of this study was to screen hexane, ethyl acetate and methanol extracts (not the oils) of seeds and fruits of *I. griffithii* for antibacterial and antifungal activities.

2. Materials and methods

2.1. Plant materials

Healthy, disease free fruits with seeds of *I. griffithii* were collected from Arunachal Pradesh, India and were identified and authenticated by the taxonomist, Department of Plant Biology and Biotechnology, Loyola College, Chennai, India, where voucher specimen (DPBB-A-42) was deposited for future reference. All the seeds were separated from fruits. Seeds and seed-free fruits were shade dried at room temperature for 15 d. The dried materials were then powdered separately and stored in the airtight container.

2.2. Preparation of crude extract

Powdered seeds (1 kg) were soaked serially in hexane (4 L), ethyl acetate (4 L), and methanol (4 L) for 72 h respectively with intermittent shaking. The solutions were filtered and the filtrates were concentrated under reduced pressure using rotary vacuum evaporator (25 °C—hexane extract; 35 °C—Ethyl acetate extract; 40 °C—Methanol extract). Finally, crude extracts were obtained and stored at 4 °C. Similarly, fruit powder (1 kg) was soaked serially in the above mentioned solvents. The extracts were obtained and stored.

The yield of seed extracts were: hexane extract (8.1%, w/w), ethyl acetate extract (9.8%, w/w) and methanol extract (11.6% w/w) and the yield of fruit extracts were: hexane extract (9.2%, w/w), ethyl acetate extract (11.2%, w/w) and methanol extract (13.8%, w/w).

2.3. Test organisms

The following test organisms were used to detect antimicrobial activity using disc diffusion method: (i) Gram positive bacterial strains (three reference cultures) including *Staphylococcus aureus* (*S. aureus*) (ATCC 25923), *Bacillus subtilis* (*B. subtilis*) (MTCC 441), and *Enterococcus feacalis* (*E. feacalis)* (ATCC 29212); (ii) Gram negative bacterial strains (nine reference cultures) including *Yersinia enterocolitica* (*Y. enterocolitica*) (MTCC 840), *Vibrio parahaemolyticus* (*V. parahaemolyticus*) (MTCC 451), *Vibrio fischeri* (*V. fischeri*) (MTCC 17380), *Enterobacter aerogenes* (*E. aerogenes*) (MTCC 111), *Escherichia coli* (*E. coli*) (ATCC 25922), *Proteus vulgaris* (*P. vulgaris*) (MTCC 1771), *Salmonella paratyphi* B (*S. paratyphi* B) , *Xanthomonas oryzae* (*X. oryzae*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) (ATCC 27853); (iii) Gram positive bacterial strains (three clinical isolates) including MRSA (Methicillin-resistant *S. aureus*) ICMR-5, MRSA (clinical pathogens) and MRSA-ATCC 29213; (iv) Gram negative bacterial strains (six clinical isolates) including *Klebsiella pneumoniae* (*K. pneumoniae*) ESBL (Extended spectrum beta-lactamase) ICMR-6, *E. coli* ESBL (clinical pathogens), *P. vulgaris*, *E. coli* (clinical pathogen), *E. coli* Cipro R ICMR-24 and *K. pneumoniae* ESBL; (v) Six fungi, *i.e.*, *Aspergillus flavus* (*A. flavus*), *Botyritis cinerea* (*B. cinerea*), *Curvularia lunata* (*C. lunata*) 46/01, *Aspergillus niger* (*A. niger*) MTCC 1344, *Trichophyton rubrum* (*T. rubrum*) 57/01 and *Trichophyton mentagrophytes* (*T. mentagrophytes*) 66/01, which were obtained as pure culture from the Department of Microbiology, Christian Medical College, Vellore, Tamil Nadu, India.

2.4. Preparation of bacterial inoculums

Bacterial inoculums were prepared by growing cells in Mueller Hinton Broth (MHB) (Himedia, Mumbai, India) for 24 h at 37 °C. These cell suspensions were diluted with sterile MHB to provide initial cell counts of about 1×10^4 CFU/ml.

2.5. Disc diffusion method

Antibacterial activity was performed using disc-diffusion method[18]. Petri plates were prepared with 20 mL of Mueller Hinton Agar (MHA) (Hi-media, Mumbai, India). The 24-hour test cultures were swabbed on the solidified media and allowed to dry for 10 min. The tests were conducted using

three different concentrations of the crude extract (1.25, 2.50 and 5.00 mg per disc). The loaded discs were placed on the surface of the medium and left for 30 min at room temperature for compound diffusion. Streptomycin (10 μg/disc) and blank discs impregnated with the respective solvents were used as positive control and negative control respectively. The plates were incubated for 18 h at 37 °C. Zone of inhibition was recorded in millimeters.

2.6. Preparation of fungal spores

The filamentous fungi were cultured on Sabouraud dextrose agar (SDA) slants at 28 °C for 10 d and the spores were collected using sterile doubled distilled water and homogenized. Yeast was cultured on Sabouraud dextrose broth (SDB) at 28 °C for 48 h.

2.7. Minimum inhibitory concentration (MIC)

The antifungal activity was performed according to the standard reference method[19]. The extracts were dissolved in water with 20% (v/v) dimethyl sulfoxide (DMSO). The initial concentration of the extract was 1 mg/mL. The initial test concentration was serially diluted two-fold. Each well was inoculated with 5 μL of fungal suspension and incubated at room temperature for 3 d. The antifungal agent fluconazole was used as positive control. MIC was defined as the lowest extract concentration showing no visible fungal growth after incubation time.

2.8. Preliminary phytochemical screening

All the extracts were subjected to preliminary phytochemical qualitative screening for the presence or absence of various primary or secondary metabolites[20].

2.9. Gas chromatography-mass spectrometry (GC-MS)

For GC-MS analysis, the samples were injected into a DP-5 column (30 m × 0.25 mm i.d with 0.25 μm film thickness), SHIMADZU Europe, GC-MS-QP 2010 model. Chromatographic conditions are as follows: helium as carrier gas, flow rate of1.50 mL/min; injector and column oven temperature 280 °C and 80 °C; injection mode, "Split" and Split ratio 1:20. Oven temperature was isothermal at 80 °C for 1 min, then increased to 300 °C, at a rate of 4 °C/min and held isothermal for 40 min. MS conditions are as follows: ionization voltage of 70 eV; ion source temperature of 200 °C; interface temperature of 240 °C; mass range of 40-1 000 mass units.

The individual peaks were identified by comparison of their retention indices to those of authentic samples, as well as by comparing their mass spectra with the Wiley 7 library (Wiley, New York, Jpan) and NIST05 (National Institute of Standards and Technology, Gaithersburg, United Sates) mass spectral database and literature.

3. Results

3.1. Antimicrobial activity

For Gram-positive and gram-negative reference cultures, the antimicrobial activity of three different solvent (hexane, ethyl acetate and methanol) extracts of seeds did not show activity against tested pathogens except that the methanol extract inhibited the growth of *B. subtilis* at 2.5 and 5.0 mg/disc concentrations (Table 1). Hexane extract of fruits of *I. griffithii* did not show activity against reference cultures (Table 2) but showed activity against *B. subtilis* at all the three concentrations. Ethyl acetate extract of fruits showed good activity against *S. aureus*, *B. subtilis*, *Y. enterocolitica*, *V. parahaemolyticus*, *S. paratyphi*, *X. oryzae* and *P. aerugenosa*. The ethyl acetate extract did not inhibit the growth of *V. fischeri*, *E. aerogens*, *E. coli* and *P. vulgaris* at all the tested concentrations. Methanol extract showed activity against only *S. aureus*, *B. subtilis* and *X. oryzae*. Other microbes were not inhibited by the methanol extract of fruits of *I. griffithii*.

For Gram-positive and gram-negative clinical isolates,

Table 1

Antimicrobial activity[a] of different solvent extracts from the seeds of *Illicium griffithii* against reference cultures.

Reference microbe	Hexane extract (mg/disc)			Ethyl acetate extract (mg/disc)			Methanol extract (mg/disc)			Streptomycin
	1.25	2.50	5.00	1.25	2.50	5.00	1.25	2.50	5.00	(25 μg/disc)
S. aureus (ATCC 25923)	–	–	–	–	–	–	–	–	–	18
B. subtilis (MTCC 441)	–	–	–	–	–	–	–	10	12	32
E. feacalis (ATCC 29212)	–	–	–	–	–	–	–	–	–	–
Y. enterocolitica (MTCC 840)	–	–	–	–	–	–	–	–	–	14
V. parahaemolyticus (MTCC 451)	–	–	–	–	–	–	–	–	–	–
V. fischeri (ATCC 17380)	–	–	–	–	–	–	–	–	–	15
E. aerogens (MTCC 111)	–	–	–	–	–	–	–	–	–	24
E. coli (ATCC 25922)	–	–	–	–	–	–	–	–	–	–
P. vulgaris (ATCC 1771)	–	–	–	–	–	–	–	–	–	–
S. paratyphi B	–	–	–	–	–	–	–	–	–	15
X. oryzae	–	–	–	–	–	–	–	–	–	17
P. aerugenosa (ATCC 27853)	–	–	–	–	–	–	–	–	–	25

[a]Antimicrobial activity is indicated by zone of inhibition (mm). –: No activity.

Table 2

Antimicrobial activity[a] of different solvent extracts from the fruits of *Illicium griffithii* against reference cultures.

Reference microbe	Hexane extract (mg/disc)			Ethyl acetate extract (mg/disc)			Methanol extract (mg/disc)			Streptomycin (25 μg/disc)
	1.25	2.50	5.00	1.25	2.50	5.00	1.25	2.50	5.00	
S. aureus (ATCC 25923)	–	–	–	12	17	24	8	12	20	18
B. subtilis (MTCC 441)	8	10	13	8	10	13	–	–	10	32
E. feacalis (ATCC 29212)	–	–	–	–	–	–	–	–	–	–
Y. enterocolitica (MTCC 840)	–	–	–	–	10	10	–	–	–	14
V. parahaemolyticus (MTCC 451)	–	–	–	–	8	10	–	–	–	–
V. fischeri (ATCC 17380)	–	–	–	–	–	–	–	–	–	15
E. aerogens (MTCC 111)	–	–	–	–	–	–	–	–	–	24
E. coli (ATCC 25922)	–	–	–	–	–	–	–	–	–	–
P. vulgaris (ATCC 1771)	–	–	–	–	–	–	–	–	–	–
S. paratyphi B	–	–	–	–	8	10	–	–	–	15
X. oryzae	–	–	–	–	10	15	–	–	10	17
P. aerugenosa (ATCC 27853)	–	–	–	8	9	12	–	–	–	25

[a]Antimicrobial activity is indicated by zone of inhibition (mm). –: No activity.

Table 3

Antimicrobial activity[a] of different solvent extracts from the seeds of *Illicium griffithii* against clinical bacterial isolates.

Clinical isolate	Hexane extract (mg/disc)			Ethyl acetate extract (mg/disc)			Methanol extract (mg/disc)			Streptomycin (25 μg/disc)
	1.25	2.50	5.00	1.25	2.50	5.00	1.25	2.50	5.00	
MRSA ICMR–5	–	–	–	–	–	–	–	–	–	–
MRSA (clinical pathogens)	–	–	–	–	–	–	8	10	10	17
MRSA ATCC–29213	–	–	10	–	–	–	–	–	–	22
K. pneumoniae ESBL ICMR–6	–	–	–	–	–	–	–	–	–	18
E. coli ESBL (clinical pathogens)	–	–	–	–	–	–	–	–	–	15
P. vulgaris	–	–	–	–	–	–	–	–	–	24
E. coli (clinical pathogens)	–	–	–	–	–	–	–	–	–	15
E. coli Cipro R ICMR–24	–	–	–	–	–	–	–	–	–	–
K. pneumoniae ESBL	–	–	–	–	–	–	–	–	–	12

[a]Antimicrobial activity is indicated by zone of inhibition (mm). –: No activity.

Table 4

Antimicrobial activity[a] of different solvent extracts from the fruits of *Illicium griffithii* against clinical bacterial isolates.

Clinical isolate	Hexane extract (mg/disc)			Ethyl acetate extract (mg/disc)			Methanol extract (mg/disc)			Streptomycin (25 μg/disc)
	1.25	2.50	5.00	1.25	2.50	5.00	1.25	2.50	5.00	
MRSA ICMR–5	10	10	10	10	10	12	–	–	–	–
MRSA (clinical pathogens)	10	17	27	10	10	13	–	–	–	17
MRSA ATCC–29213	20	20	25	–	10	12	–	–	–	22
K. pneumoniae ESBL ICMR–6	16	20	30	10	10	12	–	–	10	18
E. coli ESBL (clinical pathogens)	–	–	–	–	–	–	–	–	–	15
P. vulgaris	10	10	10	10	11	12	–	–	–	24
E. coli (clinical pathogens)	10	13	20	–	10	12	–	–	–	15
E. coli Cipro R ICMR–24	–	–	–	–	10	12	–	–	–	–
K. pneumoniae ESBL	–	–	–	–	–	–	–	–	–	12

[a]Antimicrobial activity is indicated by zone of inhibition (mm). –: No activity.

Table 5

Minimum inhibitory concentration (MIC, mg/mL of broth) of solvent extracts from fruits of *Illicium griffithii* against clinical bacterial isolates.

Clinical isolate	Hexane extract	Ethyl acetate extract	Methanol extract
B. subtilis	0.500	0.250	>5.000
MRSA ICMR-5	1.250	1.250	>5.000
MRSA (clinical pathogens)	0.625	0.625	>5.000
MRSA-ATCC 29213	0.078	1.250	>5.000
K. pneumoniae ESBL ICMR-6	0.156	0.625	2.500
P. vulgaris	0.625	0.312	>5.000
E. coli (clinical pathogen)	0.625	1.250	>5.000
E. coli Cipro R ICMR-24	>5.000	0.625	>5.000

Table 6

Antifungal activity[a] of extracts from seeds of *Illicium griffithii*.

Fungi	Hexane extract	Ethyl acetate extract	Methanol extract	Flu	Ket
A. flavus	2 000	2 000	2 000	100.0	<12.5
B. cinerea	2 000	500	1 000	100.0	<12.5
C. lunata 46/01	1 000	2 000	2 000	25.0	<12.5
A. niger MTCC 1344	2 000	2 000	2 000	<12.5	<12.5
T. rubrum 57/01	2 000	2 000	>2 000	50.0	<12.5
T. mentagrophytes 66/01	2 000	2 000	>2 000	25.0	<12.5

[a]Antifungal activity is indicated by minimum inhibitory concentration (μg/mL). Flu: Fluconazole; Ket: Ketoconazole.

Table 7

Antifungal activity[a] of extracts from fruits of *Illicium griffithii*.

Fungi	Hexane extract	Ethyl acetate extract	Methanol extract	Flu	Ket
A. flavus	2 000	2 000	>2 000	100.0	<12.5
B. cinerea	1 000	2 000	>2 000	100.0	<12.5
C. lunata 46/01	1 000	1 000	2 000	25.0	<12.5
A. niger MTCC 1344	2 000	2 000	2 000	<12.5	<12.5
T. rubrum 57/01	2 000	1 000	>2 000	50.0	<12.5
T. mentagrophytes 66/01	2 000	1 000	>2 000	25.0	<12.5

[a]Antifungal activity is indicated by minimum inhibitory concentration (μg/mL). Flu: Fluconazole; Ket: Ketoconazole.

hexane and ethyl acetate extracts of seed did not show activity against any of the tested clinical isolates (Table 3). Methanol extract showed activity against MRSA (clinical pathogens). Hexane extract of fruit showed stronger activity against *K. pneumoniae* ESBL ICMR-6, MRSA (clinical pathogens), MRSA-ATCC and *E. coli*. The growth of *P. vulgaris* was inhibited at all the tested concentrations. Ethyl acetate extract moderately inhibited the growth of *K. pneumoniae* ESBL ICMR-6 and *P. vulgaris*, *E. coli* (clinical pathogens), MRSA-ICMR-5, MRSA (clinical pathogens) and ATCC. Hexane and ethyl acetate extracts did not show activity against *E. coli* ESBL (clinical pathogens) and *K. pneumoniae* ESBL. Methanol extract did not show activity against any of the tested clinical isolates (Table 4).

MIC of active fruit extract was determined using broth microdilution method. The fruit hexane extract inhibited

Table 8

Phytochemical analysis of hexane, ethyl acetate and methanol extracts from seeds and fruits of *Illicium griffithii*.

Chemical constituent	Test	IS1	IS2	IS3	IF1	IF2	IF3
Carbohydrate	Molish's test	-	-	-	-	++	+++
	Fehlings test	-	-	-	-	++	++
	Benedict's test	-	-	-	-	+++	+++
Saponin		-	-	-	-	+	+
Glycoside	Borntrager's test	-	-	-	-	-	-
	Legals test	-	-	-	-	-	-
Cardiac Glycoside	Keller-killani test	-	-	-	-	-	-
Alkaloide test	Mayer's test	-	-	-	++	+	-
	Wagner's test	-	-	-	++	-	-
	Hager's test	-	-	-	++	++	-
Protein & amino acid		-	-	-	-	-	-
Phenol	$FeCl_3$ test	-	-	+	-	++	++
	Gelatin test	-	-	+	++	++	++
	Lead acetate test	-	-	+	++	++	++
Flavonoid	NaOH test	-	-	++	-	+	+
	NH_4OH test	-	-	++	-	++	++
	Na_2CO_3 test	-	-	+	-	+	+
	Mg turnings	-	-	+	-	+	++
Sterol		-	-	-	-	-	-
Triterpenoid/Steroid	Salkowski test	-/+	+/-	-/+++	-/+	+/-	-/-
Gum		-	-	-	-	-	-
Anthroquinone		-	-	-	-	-	-
Tanin	K_2CrO_2 test	-	-	-	-	-	-
	$FeCl_3$ test	-	-	+	++	++	++
	Lead acetate test	-	-	+	++	++	++

- = absent; + = Present; ++ = Moderate; +++ = Intense. IS1, IS2, and IS3 represent hexane, ethyl acetate and methanol extracts of seed respectively. IF1, IF2, and IF3 represent hexane, ethyl acetate and methanol extracts of fruits respectively.

Table 9

Total ionic chromatogram (GC-MS) of hexane extract of *Illicium griffithii* fruit with 70 eV using a BP-5 MS column (30 m × 0.25 mm) with helium as carrier gas.

No	Retention time (min)	Compound name	Compound nature	Molecular formula	Molecular weight	Area (%)	Retention index	Similarity index
1	6.010	Dimethyl-1,6-octadien-3-ol	Monoterpene alcohol	$C_{10}H_{18}O$	154	10.70	1 101	97
2	8.301	Alpha-terpineol	Monoterpene alcohol	$C_{10}H_{18}O$	154	0.39	1 143	97
3	11.067	1,3-Benzodioxole, 5-(2-propenyl)-	Safrole	$C_{10}H_{10}O_2$	162	1.09	1 327	96
4	12.575	o-Menth-8-ene	Sesquiterpene	$C_{15}H_{24}$	204	0.07	1 431	92
5	12.952	Alpha-cubebene	Sesquiterpene	$C_{15}H_{24}$	204	0.40	1 344	97
6	13.096	3-Allyl-2-methoxyphenol	Phenol	$C_{10}H_{12}O_2$	204	0.07	1 392	96
7	13.754	Copaene	Sesquiterpene	$C_{15}H_{24}$	154	0.79	1 221	97
8	13.880	Geraniol acetate	Ester	$C_{12}H_{20}O_2$	196	0.93	1 352	97
9	14.028	Beta-bourbonene	Sesquiterpene	$C_{15}H_{24}$	204	0.81	1 339	97
10	14.222	Beta-elemene	Sesquiterpene	$C_{15}H_{24}$	204	0.63	1 398	96
11	14.771	Alpha-gurjunene	Sesquiterpene	$C_{15}H_{24}$	204	0.43	1 419	96
12	15.073	Caryophyllene	Sesquiterpene	$C_{15}H_{24}$	204	3.74	1 494	97
13	15.337	Beta-cubebene	Sesquiterpene	$C_{15}H_{24}$	204	0.30	1 339	96
14	16.060	Alpha-humulene	Sesquiterpene	$C_{15}H_{24}$	204	0.72	1 579	94
15	16.333	Epi-bicyclosesquiphellandrene	Sesquiterpene	$C_{15}H_{24}$	204	0.24	1 435	94
16	16.913	Germacrene-D	Sesquiterpene	$C_{15}H_{24}$	204	7.83	1 339	96
17	17.426	Myristicin	Phenylpropene	$C_{11}H_{12}O_3$	192	29.20	1 516	87
18	17.882	Gamma-muurolene	Sesquiterpene	$C_{15}H_{24}$	204	1.33	1 435	92
19	18.132	6-Allyl-1,3-benzodioxol-5-ol	Phenol	$C_{10}H_{10}O_3$	178	3.42	1 547	85
20	19.604	Germacrene D-4-ol	Sesquiterpene alcohol	$C_{15}H_{26}O$	222	1.39	1 660	92
21	20.355	Methoxyeugenol	Phenol	$C_{11}H_{14}O_3$	194	2.77	1 581	92
22	21.373	5-Allyl-6-(allyloxy)-1,3-benzodioxole	Benzodioxole	$C_{13}H_{14}O_3$	218	0.63	1 705	92
23	21.439	Tau-cadinol	Sesquiterpene alcohol	$C_{15}H_{26}O$	222	0.86	1 580	85
24	21.806	Alpha-cadinol	Sesquiterpene alcohol	$C_{15}H_{26}O$	222	1.18	1 580	88
25	22.654	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol	Sesquiterpene alcohol	$C_{15}H_{24}O$	220	0.19	1 690	86
26	29.791	l-(+)-Ascorbic acid 2,6-dihexadecanoate	Fatty acid	$C_{38}H_{68}O_8$	652	0.41	4 765	91
27	30.983	Rimuene	Diterpene hydrocarbon	$C_{20}H_{32}$	272	0.40	1 926	82
28	31.925	Ar-abietatriene	Hydrocarbon	$C_{20}H_{30}$	270	0.08	2 004	82
29	32.555	1-Octadecanol	Fatty alcohol	$C_{18}H_{38}O$	270	0.08	2 053	92
30	33.753	9,12-Octadecadienoic acid (Z,Z)-	Linoleic acid	$C_{18}H_{32}O_2$	280	0.26	2 183	91
31	33.894	Oleic acid	Fatty acid	$C_{18}H_{34}O_2$	282	0.58	2 175	90

The GC/MS retention indices were calculated using a homologous series of *n*-alkanes C6-C25. Components were tentatively identified based on library and literature searches and only those components showing matches exceeding 80% were selected.

Table 10

Total ionic chromatogram (GC-MS) of ethyl acetate extract of *Illicium griffithii* fruit with 70 eV using a BP-5 MS column (30 m × 0.25 mm) with helium as carrier gas.

No	Retention time (min)	Compound name	Compund nature	Molecular formula	Molecular weight	Area (%)	Retention index	Similarity index
1	5.419	Linalool oxide (2)	Monoterpenoid	$C_{10}H_{18}O_2$	170	0.22	1 182	93
2	5.825	1-Acetoxy-2,3-dihydroxypropane	Monoacetin	$C_5H_{10}O_4$	134	4.52	1 091	94
3	6.000	Dimethyl-1,6-octadien-3-ol	Monoterpenoid alcohol	$C_{10}H_{18}O$	154	9.28	1 101	97
5	7.974	4-Terpineol	Monoterpenoid alcohol	$C_{10}H_{18}O$	154	0.12	1 137	96
6	8.188	3,7-dimethyl-1,5-octadien-3,7-diol	Aliphatic alcohol	$C_{10}H_{18}O_2$	170	0.09	1 197	88
7	8.309	Alpha-terpineol	Monoterpenoid alcohol	$C_{10}H_{18}O$	154	0.43	1 143	97
8	8.460	Pyrocatechol	Phenol	$C_6H_6O_2$	110	0.79	1 122	96
9	9.690	Acetoglyceride	Ester	$C_5H_{10}O_4$	134	1.96	1 091	95
10	11.067	1,3-Benzodioxole, 5-(2-propenyl)	Safrole	$C_{10}H_{10}O_2$	162	1.07	1 327	95
11	12.862	1,2,3-Propanetriol, diacetate	Ester	$C_7H_{12}O_5$	176	0.14	1 230	90
12	13.086	3-Allyl-6-methoxyphenol	Phenol	$C_{10}H_{12}O_2$	164	0.20	1 392	96
13	13.756	Alpha-copaene	Sesquiterpene	$C_{15}H_{24}$	204	0.44	1 221	96
14	13.878	Geranyl acetate	Ester	$C_{12}H_{20}O_2$	196	0.65	1 352	95
15	14.033	Beta-bourbonene	Sesquiterpene	$C_{15}H_{24}$	204	0.28	1 339	96
16	14.772	Alpha-gurjunene	Sesquiterpene	$C_{15}H_{24}$	204	0.22	1 419	95
17	15.059	Trans-caryophyllene	Sesquiterpene	$C_{15}H_{24}$	204	1.16	1 494	97
18	16.063	Alpha-humulene	Sesquiterpene	$C_{15}H_{24}$	204	0.26	1 579	94
19	16.646	Delta-cadinene	Sesquiterpene	$C_{15}H_{24}$	204	0.25	1 469	89
20	16.732	Gamma-muurolene	Sesquiterpene	$C_{15}H_{24}$	204	0.63	1 435	94
21	16.843	Alpha-amorphene	Sesquiterpene	$C_{15}H_{24}$	204	0.13	1 440	94
22	17.028	Alpha-guaiene	Sesquiterpene	$C_{15}H_{24}$	204	0.10	1 490	90
23	17.331	Myristicin	Phenylpropene	$C_{11}H_{12}O_3$	192	25.45	1 516	88
24	17.845	Gamma-cadinene	Sesquiterpene	$C_{15}H_{24}$	204	1.08	1 435	95
25	18.514	1-Isopropyl-4,7-dimethyl-1,2,4a,5,6,8a-hexahydronaphthalene	Sesquiterpene	$C_{15}H_{24}$	204	0.23	1 440	95
26	20.327	4-Allyl-2,6-dimethoxyphenol	Phenol	$C_{11}H_{14}O_3$	194	3.49	1 581	93
27	21.080	Diepi-alpha-cedren I	Sesquiterpene	$C_{15}H_{24}$	204	0.22	1 375	81
28	21.370	5-Allyl-6-(allyloxy)-1,3-benzodioxole	Benzodioxole	$C_{13}H_{14}O_3$	218	0.23	1 705	85
29	21.434	Torreyol	Sesquiterpene alcohol	$C_{15}H_{26}O$	222	1.09	1 580	83
30	21.801	Alpha-cadinol	Sesquiterpene alcohol	$C_{15}H_{26}O$	222	1.43	1 580	87
31	22.657	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol	Sesquiterpene alcohol	$C_{15}H_{24}O$	220	0.13	1 690	80
32	26.902	1-Hydroxy-2-(prop-2-enyl)-4,5-methylenedioxybenzene	Phenol	$C_{10}H_{10}O_3$	178	5.85	1 547	85
33	27.160	4-Allyl-2,6-dimethoxyphenol	Phenol	$C_{11}H_{14}O_3$	194	2.83	1 581	83
34	29.788	Hexadecanoic acid	Palmitic acid	$C_{16}H_{32}O_2$	256	1.96	1 968	93
35	30.517	Hexadecanoic acid, ethyl ester (CAS) ethyl palmitate	Fatty acid ethyl ester	$C_{18}H_{36}O_2$	284	0.39	1 978	81
36	33.728	9,12-Octadecadienoic acid (Z,Z)	Linoleic acid	$C_{18}H_{32}O_2$	280	1.23	2 183	92
37	33.877	Oleic acid	Fatty acid	$C_{18}H_{34}O_2$	282	1.69	2 175	90
38	34.336	Ethyl linoleate	Fatty acid	$C_{20}H_{36}O_2$	308	0.46	2 193	92
39	34.468	Ethyl oleate	Fatty acid	$C_{20}H_{38}O_2$	310	0.32	2 185	84

The GC/MS retention indices were calculated using a homologous series of *n*-alkanes C6-C25.

maximum number of tested clinical pathogens at lower test concentration (Table 5).

The MIC for antifungal activity of seed and fruit extracts was also measured using broth microdilution method. The lowest MIC value was observed in seed ethyl acetate extract against *B. cinerea* at 500 mg/mL. All other tested fungi were inhibited by seed and fruit extract of *I. griffithii* at 1 000 and 2 000 mg/mL concentrations (Tables 6 & 7).

3.2. Phytochemical analysis

The phytochemical qualitative analysis revealed the presence of phenols, tannins, flavonoids, triterpenoids, steroids, alkaloids, saponis and carbohydrates in the different extracts (Table 8).

3.3. Chemical composition by GC-MS analysis

The crude hexane and ethyl acetate extracts of *I. griffithii* fruits were studied for their chemical composition using GC-MS analysis. Thirty one components were identified in the hexane extract (Table 9). These compounds comprised mainly phenyl propenes (30.29%), phenols (28.77%), sesquiterpenes (16.79%), monoterpene alcohols (14.71%), esters (2.75%), fatty acids (0.84%), and diterpene hydrocarbons (0.40%). The major compounds were myristicin (29.20%) and linalool (10.70%). The other components including 6-allyl-1,3-benzodioxol-5-ol, caryophyllene, germacrene-D, and methoxyeugenol were found to be less than 10%. Rest of the constituents were found to be insignificant amount.

Thirty nine components were identified in the ethyl acetate extract (Table 10). These compounds consisted of mainly phenyl propenes (26.25%), phenols (13.23%), monoterpenoid alcohol (12.48%), sesquiterpenes (6.92%), fatty acids (6.37%), esters (2.75%), and monoterpenoids (0.4%). Myristicin (25.45%) and linalool (9.25%) were the major compounds. 1-Hydroxy-2-(prop-2-enyl)-4,5-methylenedioxybenzene was found to be less than 10%.

4. Discussion

The general chemical profile of the extracts, retention time, constituent name, nature, molecular formula, molecular weight, percentage area, retention index and similarity index of the constituents were summarized. From the hexane extract, a total of 31 volatile constituents were tentatively identified on the basis of their mass spectra, which were compared to those in the literature. From the ethyl acetate extract of fruit of *I. griffithii*, a total of 39 constituents were identified. Only the components showing above 80% similarity index match were selected.

The chemical composition of oils from several parts (leaves, branches, fruits and root-bark) of *I. griffithii* was investigated and reported[12,21]. Another study on the fruit oil of *I. griffithii* harvested from Vietnam reported the following compounds such as alpha-pinene, linalool, limonene and 1,8-cineole as main components[22]. Essential oils of *I. anisatum* were reported to have terpene-4-ol, a-terpineol, safrole, copaene, beta-bourbonene, delta-cadinene, germacrene D, alpha-humulene, caryophyllene, alpha-amorphene, methoxyeugenol, alpha-cadinol, 6-allyl-1,3-benzodioxol-5-ol, and abietatriene[7]. In the present study, these compounds were found either in the hexane or ethyl acetate extract of *I. griffithii*. Forty one volatile constituents were reported from the essential oil obtained from water extracts of fruit of *I. griffithii*[23]. Among the forty one, only the following compounds such as linalool oxide, linalool, delta-cadinene, alpha-cadinol, 4-terpineol, safrole, copaene, beta-bourbonene, alpha-gurjunene, caryophyllene, cadinene, muurolene and rimuene were found in the current analysis. This difference in the main components may be due to the provenance of the plant, harvest time or development stage, extraction technique, or the use of fresh or dried plant material. All of these factors influence the chemical composition and biological activity.

Numerous studies have confirmed the antimicrobial properties of the major groups of compounds obtained from different plants[24-26]. Preliminary studies have indicated that *I. verum* has antimicrobial properties[27,28]. Methanol extract of fruit of *I. verum* had antibacterial activity against *Bacillus cereus*, *Listeria monocytogenes*, *S. aureus*, *E. coli*, and *Salmonella anatum*[29]. Our study showed that methanol extract of fruits of *I. griffithii* inhibited the growth of *S. aureus* (20 mm) at 5.0 mg/disc, while ethyl acetate extract of fruits inhibited the growth of *S. aureus* (24 mm) and *P. aerugenosa* (12 mm) at 5.0 mg/disc.

Antibacterial activity of the essential oil obtained from water extract of fruits of *I. griffithii* against five strains, *viz*., *K. pnemoniae*, *P. aureginosae*, *E. coli*, *P. vulgaris* and *S. aureus* and its antifungal activity against fungi such as *A. niger*, *Penicillium* spp. were reported[23]. It inhibited *S. aureus* (14 mm) at 10 and 20 μL. Our study revealed that methanol extract of fruits of *I. griffithii* inhibited the growth of reference strain *S. aureus* (ATCC 25923) (20 mm) at 5.0 mg/disc. The hexane extract of fruits of *I. griffithii* inhibited the growth of reference strain *P. vulgaris* (10 mm) at all the tested concentrations and also the clinical isolate *E. coli* (clinical pathogens) (20 mm) at 5.0 mg/disc. The ethyl acetate extract of fruit of *I. griffithii* inhibited the growth of reference strains such as *S. aureus* (ATCC 25923) (24 mm) at 5.0 mg/disc, *P. aerugenosa* (12 mm) at 5.0 mg/disc, and also the clinical isolates like *P. vulgaris* (12 mm) at 5.0 mg/disc, *E. coli* (12 mm) at 5.0 mg/disc.

Myristicin and its antimicrobial activities were reported[30] and it is a scavenger of cancer causing compounds[31,32]. The presence of the achillin, a

sesquiterpene lactone (0.33%) with 68% similarity index, in the flower confirms and explains early reports on the toxicity of this plant.

Antimicrobial activity of seed and 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[33] like hydroxyl groups on the active constituents, because of the ability of these substances to bind to bacterial adhesions and disturb the availability of receptors on the surface. Phenols observed in this study are phenol, 2-methoxy-3-(2-propenyl)-, 6-allyl-1,3-benzodioxol-5-ol, methoxyeugenol, pyrocatechol, 3-allyl-6-methoxyphenol, 4-allyl-2,6-dimethoxyphenol, 1-hydroxy-2-(prop-2-enyl)-4,5-methylenedioxybenzene.

Terpenoids are phenolic compounds that exhibit the antimicrobial activity and mostly mono and sesquiterpenes are active against bacteria and fungi[34-42]. Linalool, a monoterpene alcohol, is reported to have a wide range of antibacterial and antifungal activity[43,44]. The mechanism of action of terpenes is not fully understood but is speculated to involve membrane disruption by the lipophilic compounds. Monoterpene alcohols observed in this study are dimethyl-1,6-octadien-3-ol (10.70%) , alpha-terpineol, and 4-terpineol. Some of the sesquiterpenes observed are bicycloelemene, alpha-cubebene, copaene, beta-bourbonene, beta-elemene, alpha-gurjunene, caryophyllene, beta-cubebene, alpha-humulene, epi-bicyclosesquiphellandrene, germacrene-D, gamma-muurolene, trans-caryophyllene, delta-cadinene, alpha-amorphene, alpha-guaiene, gamma-cadinene, 1-isopropyl-4,7-dimethyl-1,2,4a,5,6,8a-hexahydronaphthalene, and diepi-alpha-cedren I.

The crude extract from the fruits of *I. verum* inhibited the growth of six plant fungi[45]. In the present study, the hexane and ethyl acetate extracts of *I. griffithii* fruit showed MIC values against *B. subtilis* (500, 250 μg/mL) and *E. coli* (clinical pathogen) for hexane extract (0.625 mg/mL) and ethyl acetate extract (1.25 mg/mL).

The present study concludes that the antimicrobial activity of *I. griffithii* was mainly due to the presence of phenolic compounds, monoterpenes alcohols and sesquiterpenes. A thorough analysis of the results indicated that among the extracts of *I. griffithii* fruits and seed, only the ethyl acetate extract of fruit showed promising activity against tested bacteria. Chemical compositions of active extracts were analyzed using GC-MS. *I. griffithii* fruit ethyl acetate extract can be considered a potential candidate drug in the treatment of infectious diseases caused by the tested pathogenic microbes.

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References

[1] Arya V, Yadav S, Kumar S, Yadav JP. Antimicrobial activity of *Cassia occidentalis* L (leaf) against various human pathogenic microbes. *Life Sci Med Res* 2010; **2010**(9): 1-11.

[2] Singh CR. Antimicrobial effect of callus and natural plant extracts of *Premna serratifolia* L. *Int J Pharm Biomed Res* 2011; **2**(1): 17-20.

[3] Lalitha V, Raveesha KA, Kiran B. Antimicrobial activity of *Solanum torvum* Swart. against important seed borne pathogens of paddy. *Iranica J Energy Environ* 2010; **1**(2): 160-164.

[4] Hema R, Kumaravel S, Elanchezhiyan N. Antimicrobial activity of some of the South-Indian spices and herbals against food pathogens. *Global J Pharmacol* 2009; **3**(1): 38-40.

[5] Arunkumar S, Muthuselvam M. Analysis of Phytochemical constituents and antimicrobial activities of *Aloe vera* L. against clinical pathogens. *World J Agric Sci* 2009; **5**(5): 572-576.

[6] Yob NJ, Jofrry SM, Affandi MMR, Teh LK, Salleh MZ, Zakaria ZA. *Zingiber zerumbet* (L). Smith: A review of its ethnomedicinal, chemical, and pharmacological uses. *Evid Based Complement Alternat Med* 2011; **2011**: 1-12.

[7] Kim JY, Kim SS, OH TH, Baik JS, Song G, Lee NH, et al. Chemical composition, antioxidant, anti-elastase, and anti-inflammatory activities of *Illicium anisatum* essential oil. *Acta Pharm* 2009; **59**: 289-300.

[8] Kundu SR. A synopsis on distribution and endemism of Magnoliaceae s.I. in Indian subcontinent. *Thaizia J Bot* 2009; **19**: 47-60.

[9] Saha D, Sundriyal RC. Prioritization of non-timber forest produces for income generation: A participatory approach. *J Non-Timber For Pro* 2010; **17**(4): 387-394.

[10] Saha D, Sundriyal RC. Stand structure, phenology and fruit yield of *Illicium griffithii* in western arunachal Pradesh, Northeast India. *Indian J For* 2010; **33**(4): 475-488

[11] Duchok D, Kent K, Khumbongmayum AD, Paul A, Khan ML. Population structure and regeneration status of medicinal tree *Illicium griffithii* in relation to disturbance gradients in temperate broad leaved forest of Arunachal Pradesh. *Curr Sci* 2005; **89**(4): 673-676.

[12] Tam NT, An HL, Bighelli A, Muselli A, Casanova J. Advances in the chemical composition of essential oils from *Illicium griffithii* Hook. f. et Thoms from Vietnam(a). *J Essent Oil Res* 2005; **17**(1): 79-81.

[13] Forest Resources Development Division, Department of Forests Ministry of Agriculture Royal Government of Bhutan. Guidelines for resource assessment and management of *Illicium griffithii*.

[Online] Avaible from: http://www.moa.gov.bt/moa/downloads/downloadFiles/MoADownload5eg7 852br.pdf. [cited 2008 June]

[14] Sinha GN. Forest and forestry in Arunachal Pradesh. *SRFI Inf Bull* 2008; **27**: 43.

[15] Magano J. Synthetic approaches to the neuraminidase inhibitors zanamivir (Relenza) and oseltamivir phosphate (Tamiflu) for the treatment of influenza. *Chem Rev* 2009; **109**(9): 4398-4438.

[16] The alliance of Chinese American Biotechnology and pharmaceutical Associations. Trends in Bio/Pharmaceutical Industry 2009; **5**(1): 4.

[17] Ruslay SB, Hatsu M, Hing PCC, Dorji TY, Dorji S. Determination of shikimic acid content in *Illicium griffithii* from Bhutan. *J Renew Nat Res Bhutan* 2010; **6**: 120-128.

[18] Jagtap NS, Khadabadi SS, Ghorpade DS, Banarase NB, Naphade SS. Antimicrobial and antifungal activity of *Centella asiatica* (L.) Urban Umbeliferae. *Res J Pharm Tech* 2009; **2**(2): 327-330.

[19] Clinical and Laboratory Standards Institute. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi: approved standard. 2nd ed. In: CLSI document M38-A2. Wayne: CLSL; 2008.

[20] Snathi R, Lakshmi G, Priyadharshini AM, Anandraj C. Phytoscreening of *Nerium ollender* leaves and *M. charatia* leaves. *Int Res J Pharm* 2011; **2**(1): 131-135.

[21] Tam NT, An HL, Bighelli A, Casanova J. Components of *lllicium griffithii* Hook. f. et Thoms. essential oil from Vietnam using carbon-13 NMR spectroscopy. *J Essent Oil Res* 1998; **10**(4): 433-435.

[22] Dung NX, Chinh ND, Leclercq PA. Volatile constituents of the fruit oil of *lllicium griffithii* Hook. f. et Thoms. from Vietnam. *J Essent Oil Res* 1995; **7**(4): 451-452.

[23] Saraswathy A, Shakila R, Mercy Lavanya S, Arunmozhidevi A. Essential oil constituents of *Illicium griffithii* and its antimicrobial activity. *Pharmacog Mag* 2010; **6**(23): 208-211.

[24] Jamuna Bai A, Ravishankar Rai V, Pradeepa V. Evaluation of the antimicrobial activity of three medicinal plants of south India. *Malaysian J Microbiol* 2011; **7**(1): 14-18.

[25] Bibi Y, Nisa S, Chaudhary FM, Zia M. Antibacterial activity of some selected medicinal plants in Pakistan. *BMC Complement Altern Med* 2011; **11**: 52.

[26] Bhaskarwar B, Itankar P, Fulke A. Evaluation of antimicrobial activity of medicinal plant *Jatropha podagrica* (Hook). *Roumanian Biotech Lett* 2008; **13**(5): 3873-3877.

[27] Chouksey D, Sharma P, Pawar RS. Biological activities and chemical constituents of *llicium verum* Hook fruits. *Der Pharm Sin* 2010; **1**(3): 1-10.

[28] Huang Y, Zhao J, Zhou L, Wang J, Gong Y, Chen X, et al. Antifungal activity of the essential oil of *Illicium verum* fruit and its main component trans-anethole. *Molecules* 2010; 15(11): 7558-7569.

[29] Shan B, Cai YZ, Brooks JD, Corke H. The *in vitro* antimicrobial activity of dietary spice and medicinal herb extracts. *Int J Food Microbiol* 2007; 117(1): 112-119.

[30] Gerige SI, Gerige MKY, Rao M. GC-MS analysis of *Nigella sativa* seeds and antimicrobial activity of its volatile oil. *Braz Arch Biol Technol* 2009; 52(5): 1189-1192

[31] John Zachariah T, Leela NK, Maya KM, Rema J, Mathew PA. Chemical composition of leaf oils of *Myristica beddomeii* (King), *Myristica fragrans* (Houtt.) and *Myristica malabarica* (Lamk.). J Spices Aromatic Crops 2008; **17**(1): 10-15.

[32] Stefano VD, Pitonzo R, Schillaci D. Antimicrobial and antiproliferative activity of *Athamanta sicula* L. (Apiaceae). *Pharmacog Mag* 2011; **7**(25): 31-34.

[33] Gordana SC, Jasna MC, Sonja MD. Antioxidant potential, lipid peroxidation inhibition and antimicrobial activities of *Satureja montana* L. subsp. kitaibelii extracts. *Int J Mol Sci* 2007; 8: 1013-1027.

[34] Manimaran S, Dhanabal P, Nanjan J, Suresh B. Chemical composition and antimicrobial activity of the volatile oil of the cones of *Cupressus torulosa* D. DON from Nilgiris, India. *Asian J Trad Med* 2007; **2**(6): 206-211.

[35] Bhagavathy S, Sumathi P, Bell IJS. Green algae *Chlorococcum humicola*-a new source of bioactive compounds with antimicrobial activity. *Asian Pac J Trop Biomed* 2011; **1**(Suppl 1): S1-S7.

[36] SD Karou, F Djigma, T Sagna, C Nadembega, M Zeba, A Kabre, et al. Antimicrobial resistance of abnormal vaginal discharges microorganisms in Ouagadougou, Burkina Faso. *Asian Pac J Trop Biomed* 2012; **2**(4): 294-297.

[37] Prasad TNVKV, Elumalai EK. Biofabrication of Ag nanoparticles using *Moringa oleifera* leaf extract and their antimicrobial activity. *Asian Pac J Trop Biomed* 2011; **1**(6): 439-442.

[38] Kamazeri TSAT, Samah OA, Taher M, Susanti D, Qaralleh H. Antimicrobial activity and essential oils of *Curcuma aeruginosa*, *Curcuma mangga*, and *Zingiber cassumunar* from Malaysia. *Asian Pac J Trop Med* 2012; **5**(3): 202-209.

[39] Ponnusamy P, Natarajan V, Sevanan M. *In vitro* biofilm formation by uropathogenic *Escherichia coli* and their antimicrobial susceptibility pattern. *Asian Pac J Trop Med* 2012; **5**(3): 210-213.

[40] Taher M, Susanti D, Rezali MF, Zohri FSA, Ichwan SJA, Alkhamaiseh SI, et al. Apoptosis, antimicrobial and antioxidant activities of phytochemicals from *Garcinia malaccensis* Hk.f. *Asian Pac J Trop Med* 2012; **5**(2): 136-141.

[41] Selvaraj N, Lakshmanan B, Mazumder PM, Karuppasamy M, Jena SS, Pattnaik AK. Evaluation of wound healing and antimicrobial potentials of *Ixora coccinea* root extract. *Asian Pac J Trop Med* 2011; **4**(10): 959-963.

[42] Nain P, Kumar A, Sharma S, Nain J. *In vitro* evaluation of antimicrobial and antioxidant activities of methanolic extract of *Jasminum humile* Leaves. *Asian Pac J Trop Med* 2011; **4**(10): 804-807.

[43] Dayangac A, Bahsi M, Ozkaya A, Yilmaz O. Linalool improve biochemical damage and fatty acids composition of testes on fasting male rats. *J Anim Vet Advances* 2011; **10**(9): 1232-1238.

[44] Josphat CM, Joyce JK, Nicholas MK, Tiffany PH. Chemical composition and antimicrobial activity of the essential oil of *Satureja biflora* (Lamiaceae). *Bull Chem Soc Ethiop* 2007; **21**(2): 249-254.

[45] Park IK, Kim J, Lee YJ, Shin SC. *In vivo* fungicidal activity of medicinal plant extracts against six phytopathogenic fungi. *Int J Pest Manage* 2008; **54**(1): 63-68.