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Antimicrobial and Cytotoxic Activities of Root Extracts of *Piper Chaba*

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Abstract

Antibacterial and antifungal properties of petroleum ether, chloroform, ethyl acetate and methanol extracts of *Piper chaba* (Choi) roots were studied by disc diffusion method and these activities were compared with primary standard drugs Kanamycin and Nystatin, respectively. The extracts were found to exhibit promising antibacterial and antifungal properties against Gram-positive, Gram-negative bacteria and fungi. The extracts were also studied for their cytotoxic activities by brine shrimp lethality bioassay, where gallic acid was used as primary standard. It was observed that the petroleum ether extract was potent cytotoxic with the LC_{50} value of 0.95 µg/ml against *Artemia salina* (L). The essential oils of the petroleum ether extract of *Piper chaba* roots were analysed by GC/MS. It was observed that most of the compounds were sesquiterpenes, some were long chain fatty acids and some were monoterpenes and alkaloids. Seventeen compounds were identified from the GC/MS analysis.

Keywords: Piper chaba, antibacterial activity, antifungal activity, cytotoxicity.

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1. Introduction

The plant *Piper chaba*, which is called Choi locally, belongs to the family piperaceae. The members of this family are claimed to have economic as well as medicinal importance. This family has also found a wide range of applications in the traditional pharmacopoeia of several cultural groups. It is renowned in the Indian Ayurvedic system of medicine, in the folklore of Africa, Latin America and the West Indies as well as in the Chinese herbal medicines. The plant *Piper chaba* is also an important member of this family. The plant *Piper chaba* is an annual/perennial shrub, cultivated in India, Malaysia and Bangladesh. In Bangladesh and India, the decoction of the roots of *Piper chaba* Hunter (Fam: Piperaceae) is used for colic pain, dyspepsia and gastralgia [1, 2]. Essential oils composition study on the *Piper* species have shown the presence of elemol (11.5%) in *Piper nigrum*, β-caryophyllene (13%) in *Piper attenuatum*, β-cubebene (10%) in *P.*

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cubeba, safrole (39.9%), eugenol (9.0%), allo-pyrocatechol mono acetate (8.5%), terpinen-4-ol (6.3%) and chavibetol in *Piper betel* leaf by GC and GC/MS [3]. *P.nigrum* oil, fruit oil of *Piper chaba* and *P. longum* have been found to contain few monoterpene hydrocarbons, a moderate content of sesquiterpenes and high content of aliphatic hydrocarbons by GC and GC/MS analysis [4]. Biological investigations on *Piper chaba* have shown that chloroform and methanol extracts of *Piper chaba* show anti-amoebic and anti-giardial activities against *Entamoeba histolytica* (IC_{50} value of 71.4 µg/ml) and *Giardia intestinalis* (IC_{50} value of <100 µg/ml) [5, 6]. Extracts of *Piper chaba* have also been found to exhibit anti-diarrhoeal and diuretic activities [7].

Recently, it has been reported that amides from *Piper chaba* significantly inhibit ethanol and indomethacin induced gastric lesions at a dose of 25 mg/kg after oral administration [8]. However, there is no report on chemical or biological investigations on the roots of this plant. In this paper we report antimicrobial and cytotoxic activities of the crude extracts of the roots of *Piper chaba* and the compositions of the essential oils of petroleum ether extract.

2. Materials and Methods

2.1. *Plant collection and extraction*

Roots of *Piper chaba* were collected from the Bagerhut District, Bangladesh. The plant was botanically identified by Prof. A. T. M. Naderuzzaman, Department of Botany, University of Rajshahi. A voucher specimen was deposited in Bangladesh National Herbarium (Voucher No. 30276).

The extraction was carried out by soaking the dried, grounded roots (1 kg) of *Piper chaba* in 5 liter rectified spirit for two weeks at room temperature. Evaporation of the solvent under reduced pressure afforded a semi-solid mass (40 gm). The crude extract was successively partitioned with petroleum ether, chloroform, ethyl acetate and finally with methanol. Solvents of these extracts were concentrated separately by evaporation under reduced pressure below 50 ºC, which yielded 10.0 g, 12.3 g, 1.2 g and 5.6 g of the dried extracts, respectively.

2.2. *Test organisms*

Four Gram-positive bacteria (*Bacillus subtilis, Bacillus megaterium, Staphylococcus aureus, Strep. β-haemolyticus*) and six Gram-negative bacteria (*Escherichia coli, Salmonella typhi, Shigella dysenteriae, Shigella shiga, Klebsiella spp., Shigella boydii*) were used for antibacterial activity study, four fungi (*Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus, Candida albicans*) were used for antifungal activity study and a simple zoological organism (brine shrimp nauplii) was used for cytotoxicity study. All organisms were collected from the stock culture of the Institute of Nutrition and Food Sciences, University of Dhaka, Bangladesh.

2.3. *Antibacterial, antifungal and cytotoxicity studies*

Antibacterial and antifungal activity studies were carried out by disc diffusion method [9, 10] and cytotoxicity was studied by brine shrimp lethality bioassay [11]. Standard antibiotic disc of Kanamycin (K-30 µg/disc) was used as the standard reference drug for antibacterial assay, Nystatin (50 µg/disc) was used for antifungal activity study and gallic acid was used as a standard reference for cytotoxicity study [12].

2.4. *Chemical analysis by GC/MS*

The essential oils were examined by ISO-ext method in Agilent, 6890 series-GC system (made in USA). ISO-ext method is a long routine method to determine non-polar to polar compounds in a mixture. In this analysis, total running time was 73 min from 50°C (1 min. hold) to maximum 300°C (10 min. hold) and ramp time was 4 min. Petroleum ether extract (1 gm) of *Piper chaba* root was dissolved in small amount of acetone and shaken in UV-water bath. Then acetone soluble fraction was collected and dried off by Nitrogen gas to afford an oily mass for the GC/MS analysis. 5 mg of acetone soluble fraction was dissolved in 1 ml of acetone. For this analysis, 1 µl of samples was injected in split mode and selective mass detector with helium gas at 1.6 ml/min under 31.7 psi pressures and AV with 35 cm/sec were used.

Table 1. Antibacterial activity of different extracts of the roots of *Piper chaba*.

	Diameter of zone of inhibition (mm)								
	PE (µg/disc)		CE (µg/disc)		EE (µg/disc)		ME (µg/disc)		Kanamycin (µg/disc)
Bacteria	50	200	50	200	50	200	50	200	30
Gram-positive									
Bacillus subtilis	9	16	9	17	14	30	15	28	24
Bacillus megaterium	10	15	9	14	15	28	17	30	28
Staphylococcus aureus	9	18	11	17	15	30	18	28	27
Strep. β-haemolyticus	11	17	-	19	16	32	16	27	26
Gram-negative									
Escherichia coli	8	14	9	16	15	30	16	29	28
Salmonella typhi	9	13	10	19	14	26	17	27	27
Shigella dysenteriae	10	14	-	17	16	34	15	31	29
Shigella shiga	10	15	9	18	13	29	16	25	28
Shigella boydii	9	16	11	19	15	31	16	29	27
Klebsiella spp.	-	12	-	15	16	28	17	27	26

PE - Petroleum ether extract, CE - Chloroform extract, EE - Ethyl acetate extract,
ME - Methanol extract, (-) - no inhibition.

3. Results

The results of antibacterial test of various fractions of the extracts are presented in Table 1. The antibacterial activities of these extracts are compared to standard drug Kanamycin. It can be inferred from the table that the extracts, when compared to Kanamycin, show moderate activity against various types of bacteria. However, the pure compound(s) might show even higher antibacterial activities than Kanamycin. Table 2 shows antifungal activity of the crude extracts. In this case our results have also been compared to standard drug Nystatin. The extracts show antifungal property but the extent of inhibition is lower than that of Nystatin, even with higher doses.

Table 2. Antifungal activity of different extracts of the roots of *Piper chaba*.

Test Fungus	Diameter of the zone of inhibition (mm)				
	PE 200 µg/disc	CE 200 µg/disc	EE 200 µg/disc	ME 200 µg/disc	Nystatin 50 µg/disc
Aspergillus fumigatus	12	14	8	10	25
Aspergillus niger	11	13	8	-	24
Aspergillus flavus	10	10	9	8	20
Candida albicans	12	13	10	10	15

PE - Petroleum ether extract, CE - Chloroform extract,
EE - Ethyl acetate extract, ME - Methanol extract, (-) - no inhibition.

Table 3 shows brine shrimp lethality bioassay of various fractions of the extracts and a comparison has been made against a standard reference drug gallic acid. It has been found that petroleum ether, chloroform and ethyl acetate extracts were very potent cytotoxic (LC_{50} values were less than 4.5µg/ml) in comparison to gallic acid (LC_{50} value was 4.5 µg/ml).

Table 3. Cytotoxicity test of root extracts of *Piper chaba*.

Extracts	LC_{50} (µg/ml)	95% confidence limit (µg/ml)		Regression equation	λ^2 (df)
		upper	lower		
PE	0.95	1.58	1.57	Y = 2.88 + 2.16 X	0.83 (2)
CE	1.53	0.87	2.71	Y = 3.10 + 1.59 X	0.06 (2)
EE	4.22	1.99	8.98	Y = 2.89 + 1.30 X	0.25 (3)
ME	14.21	8.41	23.99	Y = 2.97 + 1.75 X	3.23 (3)
Gallic acid	4.53	3.33	6.15	Y = 3.93 + 1.62 X	1.25 (2)

PE - Petroleum ether extract, CE - Chloroform extract.
EE - Ethyl acetate extract, ME - Methanol extract, (-) - no inhibition.
λ^2 - Chi-square value, df - Degree of freedom.

Gas chromatogram of the essential oils of petroleum ether extract of *Piper chaba* roots are shown in Fig. 1. From the GC/MS spectrum, a total of 44 peaks were identified. Some peaks were broad or mixture and some were very small which were not considered in analysis. Among the 44 peaks, the highest peak at retention time 52.14 min. possesses a basic signal at 152 in mass spectrum. It might be tri-beta-ketal skeleton compounds but could not be identified because of its 25% match with the existing library data. Out of 44

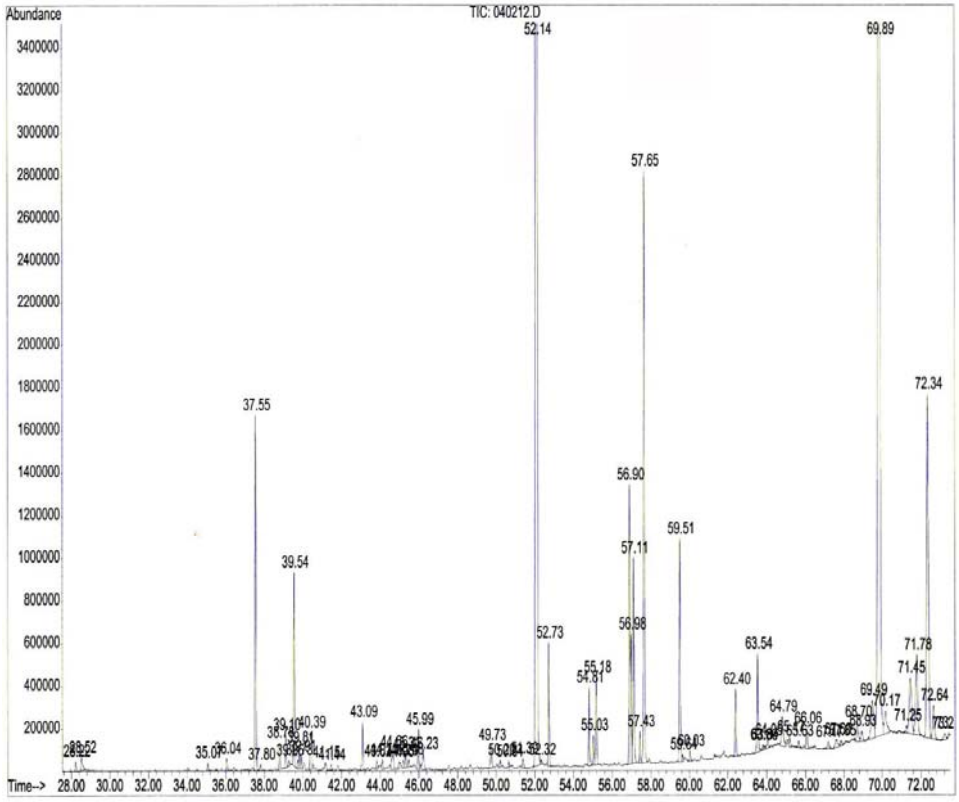

Fig. 1. Gas chromatogram of the essential oils of petroleum ether extract of *Piper chaba* roots.

peaks, 24 showed more than 80% match with the Wiley library data. The structures of only 17 compounds, which match more than 90%, were confirmed. Most of the compounds were sesquiterpenes with molecular mass of 204, some were long chain fatty acids and some might be monoterpenes and alkaloids. At the early middle of the GC/MS spectra most of the peaks were known corresponding to the oily components but at the end of the spectra there were mainly unknown compounds. Chemical profile of the acetone soluble fraction of the petroleum ether extract of *Piper chaba* root by GC/MS is given in Table 4.

Table 4. Chemical profile of the acetone soluble fraction of petroleum ether extract of *Piper chaba* roots by GC/MS.

Peak at retention time (min.)	Probable mass	Possible compounds	¾Matching with Wiley library
35.07	149	Heliotropine	97
36.04	204	β-Elemene	99
37.55	204	β-Caryophyllene	99
37.80	204	Germacrene-D	97
38.78	204	α-Humulene	98
39.10	204	α-Amorphene	98
39.26	204	(-)-Isoledene	93
39.54	204	Germacrene-D	99
39.73	204	Naphthalene	97
39.81	204	α-Muurolene	98
39.91	204	(+)-Aromadendrene	96
40.39	204	δ-Cadinene	98
41.15	204	(-)-Aromadendrene	90
41.44	204	Elemol	83
43.09	161	Caryophyllene oxide	93
43.82	220	Cyclopropazulen-7-ol	46
44.11	220	Spathulenol	89
44.66	204	Germacrene-D	86
44.96	222	T-Muurolol	86
45.20	204	γ-Gurjunene	83
45.31	177	1H-Indole-3-carboxylic acid	41
45.48	204	Thujopsene	55
45.99	162	Cyclohexane compound	35
49.73	182	Flopropione	43
50.20	241	Ethyl heptadecanoate	68
51.39	192	1,8-Nonadiene	38
52.14	152	Trimethyl-2-cyclohexene-1,4 dione	25
52.73	284	Hexadecanoic acid ethyl ester	98
54.81	166	1,2-Benzenediol	25
55.18	194	Benzoic acid	47
56.90	308	Ethyl linoleate	99
65.98	251	6-Methoxy-2′-nitroaurone	40
57.43	312	Ethyl stearate	98
57.65	235	N-Cyclodecylidene	30
59.51	151	Pyridine,4-butyl-l-oxide	59
62.40	207	4-Quinolinol	38
63.54	325	N-Methyl-2-phenyl ethylamine	59

(Table 4 continued)

Peak at retention time, (min.)	Probable mass	Possible compounds	% Matching with Wiley library
64.79	330	Hexadecanoic acid	81
66.06	150	Thymol	38
69.49	264	9,12-Octadecadienal	92
69.89	285	Δ 2-1,2,4-Triazoline-5-one	43
71.45	282	2,3-Biphenylene	25
71.78	152	2-Benzofuranone	27
72.34	135	Benzothiazole	64

4. Discussion

The ethyl acetate extract of *Piper chaba* root shows a significant antibacterial activity, followed by methanol extract against both Gram-positive and Gram-negative bacteria. This result suggests that the root extracts of *Piper chaba* can be used for the treatment of fever, bronchitis and tuberculosis. The petroleum ether and chloroform extracts show moderate antibacterial activity but they exhibit very potent cytotoxic property with LC_{50} values of 0.95 and 1.53 µg/ml, respectively, compared to standard gallic acid. This result supports the use of *Piper chaba* as anthelmintic in traditional medicine. All extracts show moderate antifungal activity. From GC/MS analysis, it is clear that the oily mass of petroleum extract of *Piper chaba* (root) possesses some sesquiterpenes plus some intermediate polar compounds which might be interesting for further isolation and investigation of biological activity.

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