

Chemical profiling, antibacterial and antiparasitic studies of *Imperata cylindrica*

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ABSTRACT

Imperata cylindrica is a common grass with little known medicinal properties. The underground part (comprising rhizome with roots) is used by the Mizo people for the treatment of microbial and intestinal worm infections. To understand the chemical and pharmacological properties of the plant part, a methanol extract was prepared in a Soxhlet apparatus, and the extract was concentrated using a vacuum rotary evaporator. Chemical analysis was done using gas chromatography-mass spectrometry. Antibacterial activity was tested by Kirby–Bauer test and antiparasitic activity by survival test *in vitro*. Fourteen volatile compounds were detected; out of which the most abundant were *n*-hexadecanoic acid and (*Z*)-18-octadec-9-enolide. Some compounds detected including 2-methoxy-4-vinylphenol, 6-methylenebicyclo [3.2.0] hept-3-en-2-one and phenol,2,4-bis (1,1-dimethylethyl) are already known biologically active compounds. The plant extract was effective against all bacteria tested that included Gram-negative bacteria such as *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, and a Gram-positive bacterium *Bacillus subtilis*. It was also effective against two avian intestinal worms such as the tapeworm *Railletina echinobothrida* and the roundworm *Ascaridia galli*. These findings reveal the importance of this plant as a source of therapeutic compounds.

INTRODUCTION

A major conundrum in modern medicine and pharmacology is the widespread emergence and evolution of drug resistance among clinically important pathogens and threatens human and veterinary health, food security, and socio-economic conditions (Caniça *et al.*, 2019; Idris *et al.*, 2019). In terms of prevalence and diversity of the infections, bacteria and helminth parasites are at the forefront of this drug resistance dilemma. In fact, multi-drug resistance in bacteria has become a major factor in increased morbidity and mortality worldwide (Bogoch *et al.*, 2019; Jenkins *et al.*, 2017). Anthelmintic resistance is specifically pervasive among helminth parasites of livestock animals. Virtually all veterinary helminths have developed resistance to all available anthelmintic drugs (Brooker, 2018; Moser *et al.*, 2019). It is for these reasons that recent vocal advocates for the pressing need for

new and alternative medications are supportively justified (Clarke *et al.*, 2018; Willing *et al.*, 2018).

Imperata cylindrica (L.) Rauschel is a perennial and rhizomatous grass in the family Poaceae. It has a thin and lanceolate leaf, and a relatively short stem. The underground parts consist of rhizome and tiny roots that emerge from nodes on the rhizome. The plant is used in different traditional medicines of Southeast Asia for the treatment of various diseases. The flowers and roots have been used as antibacterial, diuretic, skin softening (emollient), antipyretic (febrifuge), salivating (sialagogue), anticoagulant (styptic), and soothing (tonic) agents (Townson, 1991). The roots are specifically used for the treatment of jaundice, edema, and hematological disorders such as blood urine (hematuria), blood vomit (hematemesis), and nosebleed (epistaxis). Bioactive compounds isolated from the leaves are also shown to have neuro-protective (Yoon *et al.*, 2006) and vasodilative effects (Matsunaga *et al.*, 1994).

To the Mizo people of India and Myanmar, the underground part (rhizome-root extract) is a familiar remedy for bacterial infections and has been used in the treatment of dermal wound, cholera, dysentery, and diarrhea. It is also known to be a good antifungal agent for ringworms and other

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dermatitis (Sawmliana, 2013). The most unique application is for deworming intestinal helminthiasis. The underground parts are crushed, and the juice is directly consumed (Lalthanpuii *et al.*, 2018). The importance of this antiparasitic activity is that, while most anthelmintic compounds or drugs are helminth-specific, the plant extract is equally used for both tapeworm and roundworm species. Therefore, in the light of its medicinal properties, it is worthwhile to perform chemical profiling and test its antibacterial and antiparasitic potentials.

MATERIALS AND METHODS

Plant material

Imperata cylindrica was collected from Ngopa (located between 23.8861° latitude north and 93.2119° longitude east), a village in Champhai district, Mizoram, India. The specimen was identified at the Botanical Survey of India, Shillong, Meghalaya, and a voucher herbarium (PUC-I-2018-01) is maintained at Pachhunga University College, Aizawl, Mizoram. The underground parts consisting of rhizome and roots were selected and dried in shade at room temperature ranging from 21°C to 27°C.

Preparation of plant extracts

The dried specimens were pulverized to powder in an electric grinder. Methanol extract was prepared in a 5-liter Soxhlet apparatus. The extract was concentrated by recovering the methanol in a vacuum rotary evaporator (Buchi Rotavapor® R-215). The final plant extract was semi-solid mass and was kept at 4°C until further use.

Gas chromatography-mass spectrometry (GC-MS) analysis

The methanol extract of *I. cylindrica* was analyzed for chemical identification using the GC-MS system (Thermo Scientific TRACE™ 1300 ISQ™ LT). Sample was dissolved in acetonitrile. A non-polar column used was TR-5MS (260F142P) with a dimension of 30 m × 0.25 mm × 0.25 µm and film thickness of 0.25 µm. The injector port temperature was set at 250°C. The oven temperature was set at 70°C for 2 minutes and gradually raised at an increment of 10°C up to 250°C. Helium was used as the carrier gas and set at a constant flow rate of 1 ml/minute. The sample was injected at a volume of 1 µl in the split mode at a splitting ratio of 1:50. The mass spectrometer was run with an electron energy of 70 eV for ionization. Ion source and transfer line temperature were set at 250°C. Thermo Scientific™ Xcalibur™ software was used for data generation. The total running duration was 55 minutes. Compounds were identified based on retention time, mass spectra, and molecular weight from libraries of Wiley Registry™ and the National Institute of Standards and Technology database.

Antibacterial activity

The antibacterial activity of the plant extract was studied using the Kirby–Bauer test. An aerobic Gram-negative bacterium *Pseudomonas aeruginosa* ATCC® 10145™ and an anaerobic Gram-negative bacterium *Klebsiella pneumoniae* ATCC® 10031™, and an aerobic Gram-positive bacterium *Bacillus subtilis* ATCC® 11774™ were used. The bacteria were grown in culture dishes containing Mueller–Hinton agar. The plant extracts were prepared in two concentrations, namely, 10 and 20 mg/ml.

They were impregnated on absorbent disks (Whatman Antibiotic Assay Disks). Negative control contained only the bacteria in agar medium. 20 mg/ml of ceftriaxone was used as a standard positive antibiotic. The assays were performed in triplicates. The culture dishes were maintained at 37°C ± 1°C in a microbiological incubator for 20 hours. After incubation, the sizes of the inhibition zones were measured.

Antiparasitic test

Antiparasitic test was performed on two intestinal worms, namely, a tapeworm *Railletina echinobothrida* Mégnin, 1880, and a roundworm *Ascaridia galli* Schrank, 1788. Ethical clearance for the experiment was issued by Pachhunga University College (PUC-IAEC-2016-Z2 of 10/08/2016). The parasites were collected from a freshly sacrificed local fowl, *Gallus gallus domesticus* Linnaeus, 1857. One hour before the experimental assay, solutions of incremental concentrations such as 5, 10, and 20 mg/ml of the plants extract were prepared in culture plates by dissolving them in 0.9% neutral phosphate-buffered saline (PBS) supplemented with 1% dimethylsulfoxide (DMSO). Corresponding concentrations were also prepared for albendazole as standard references. Control experiment consisted only of PBS with DMSO. The parasites were incubated at 37°C ± 1°C in each of the test media. Batches of three worms were selected for each test, and each test was further performed in triplicates. Efficacy was assessed in terms of survival in the culture media. Death was defined as no further motor activity even after stimulation with tepid PBS (45°C). The times of death were recorded, and data were generated as statistical means ± standard deviation. Significance of the antiparasitic activity was determined using unpaired Student's *t*-test, and the level of significance was considered when the *p*-value was <0.05.

RESULTS

Extraction and detection of phytochemicals

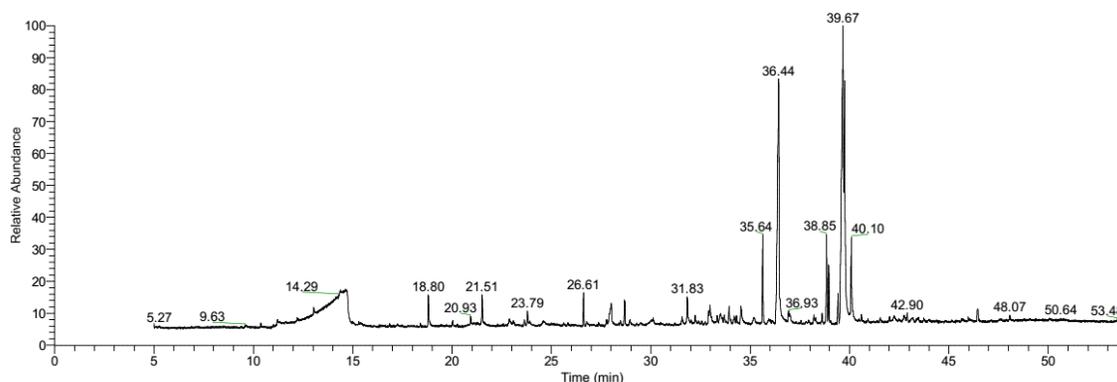
The underground parts of *I. cylindrica* yielded 19.75 g of methanol extract from 110 g of the plant sample, i.e., with an extractive value of 17.95%. Standard chemical detection using GC-MS revealed important compounds in the methanol extract of *I. cylindrica* as shown in Table 1. Compounds were identified from the chromatogram as given in Figure 1. The presence of 14 volatile compounds was established, while compounds with low probabilities were excluded. *n*-Hexadecanoic acid and (*Z*)-18-octadec-9-enolide were the most abundant at 86.2% each. In addition, methyl 9-cis, 11 trans-octadecadienoate (36.8%), hexadecanoic acid, methyl ester (35.8%), octadecanoic acid (35.8%), 6-methylenebicyclo [3.2.0] hept-3-en-2-one (17%), phenol,2,4-bis (1,1-dimethylethyl) (16.8%), 2-methoxy-4-vinylphenol (16.4%), and (*E*)-4-(3-hydroxyprop-1-en-1-yl)-2-methoxyphenol (15.8) were the major constituents.

Antibacterial activity

Ceftriaxone at 20 mg/ml was effective on the growth inhibition of all bacteria tested, producing inhibition zones of 13 mm on *B. subtilis*, 14 mm on *P. aeruginosa*, and 12 mm on *K. pneumoniae*. The methanol extract of *I. cylindrica* was also effective on all bacteria tested. At 10 and 20 mg/ml, the plant

Table 1. Chemical compounds identified from the methanol extract of *I. cylindrica* underground parts using GC-MS.

Sl. no	Retention time	Compound	Formula	Molecular weight (Da)	Abundance (%)
1.	18.80	6-Methylenebicyclo [3.2.0] hept-3-en-2-one	C ₈ H ₈ O	120	17.0
2.	21.51	2-Methoxy-4-vinylphenol	C ₉ H ₁₀ O ₂	150	16.4
3.	23.79	Benzaldehyde, 3-(chloroacetoxy)-4-methoxy	C ₁₀ H ₉ ClO ₂	228	11.6
4.	24.59	Cyclopropanetetradecanoic acid, 2-octyl, methyl ester	C ₂₆ H ₅₀ O ₂	394	8.6
5.	26.61	Phenol,2,4-bis (1,1-dimethylethyl)	C ₁₄ H ₂₂ O	206	16.8
6.	31.83	(E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol	C ₁₀ H ₁₂ O ₃	180	15.8
7.	35.64	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270	35.8
8.	36.44	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	86.2
9.	36.93	L-Methionyl-D-glutaminy-L-methionyl-L-asparaginy-L-lysyl-L-valyl-L-leucyl-D-α-aspartyl-L-serine	C ₄₃ H ₇₆ N ₁₂ O ₁₅ S ₂₁	1064	11.8
10.	38.85	Methyl 9-cis, 11 trans-octadecadienoate	C ₁₉ H ₃₄ O ₂	294	36.8
11.	39.67	(Z)-18-Octadec-9-enolide	C ₁₈ H ₃₂ O ₂	280	86.2
12.	40.10	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	284	35.8
13.	42.90	Octadecanoic acid, ethyl ester	C ₂₅ H ₄₂ O ₂	374	11.4
14.	48.07	2,4,6,8,10-Tetradecapentanoic acid, 9a-(acetyloxy)-1a,1b,4,4a,5,7a,7b,8,9a-decahydro-4a,7b-dihydroxy-3-(hydroxymethyl)-1, 1,6,8-tetramethyl-5-oxo-1H-cyclopropa[3,4]benz[1,2-e] azulen-9yl ester, [1aR-(1aa,1ba,4aa,7aa,7ba,8a,9a,9aa)]-	C ₃₆ H ₄₆ O ₈	606	10.4

**Figure 1.** GC-MS chromatogram of the methanol extract of *I. cylindrica* underground parts.

extract produced inhibition zones of 9 and 10 mm on *B. subtilis*, 7 and 8 mm on *P. aeruginosa*, and 6 and 8 mm on *K. pneumonia*, respectively. Thus, the relative susceptibility of the bacteria species for the plant extract was in an order *B. subtilis* > *P. aeruginosa* > *K. pneumonia*.

Antiparasitic activity

The methanol extract of *I. cylindrica* was effective against both the tapeworms and roundworms. As given in Table 2, control tapeworms survived up to 74.03 ± 1.89 hours. Albendazole was highly effective and killed *R. echinobothrida* in 14.99 ± 0.43 , 12.07 ± 0.49 , and 8.99 ± 0.45 hours at the concentrations of 5, 10, and 20 mg/ml, respectively. At similar concentrations, *I. cylindrica* extract took 60.69 ± 4.81 , 48.19 ± 4.89 , and 30.58 ± 3.15 hours to completely kill the worms. *A. galli* survived much longer, up to 187.01 ± 6.77 hours in the plant extract (Table 3). In albendazole, they survived for 56.94 ± 1.76 , 18.01 ± 2.73 , and 15.97 ± 1.99 hours at 5, 10, and 20 mg/ml, respectively. *Imperata cylindrica* extract was effective only at 10 and 20 mg/ml.

DISCUSSION

GC-MS analysis of the underground parts of *I. cylindrica* revealed the presence of interesting volatile compounds. Of the different compounds detected from *I. cylindrica*, some are already known to have important biological effects. For example, 2-methoxy-4-vinylphenol has been isolated from pine (*Pinus* species) needles and is demonstrated to have potent anticarcinogenic activity by specifically blocking the hyper-phosphorylation of retinoblastoma protein *in vitro* (Jeong and Jeong, 2010). It also promotes anti-inflammatory response suppression of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and mitogen-activated protein kinase (MAPK) activation, and acetylation of histone H3 (Jeong *et al.*, 2011). 6-Methylenebicyclo [3.2.0] hept-3-en-2-one is also isolated from *Allium tuberosum* and is shown to be an important defensive molecule for the plant against infection with the root-knot nematode, *Meloidogyne* species (Huang *et al.*, 2016). Phenol,2,4-bis (1,1-dimethylethyl) is reported from avocado (*Persea americana*) and acts as an effective antifungal compound against fungal pathogens such as *Aspergillus* and *Phytophthora cinnamomi* (Rangel-Sánchez *et al.*, 2014).

Table 2. Efficacy of the methanol extract of *I. cylindrica* underground parts on the tapeworm, *R. echinobothrida*. ($n = 6$).

Treatment	Dose (mg/ml)	Survival time (hour) in mean \pm standard deviation (SD)	<i>t</i> value	<i>t</i> critical value
Control	0	74.03 \pm 1.89	NA	NA
Albendazole	20	08.99 \pm 0.45*	81.85	2.23
	10	12.07 \pm 0.49*	77.66	2.23
	5	14.99 \pm 0.43*	74.53	2.23
<i>I. cylindrica</i>	20	30.58 \pm 3.15*	28.99	2.23
	10	48.19 \pm 4.89*	12.06	2.23
	5	60.69 \pm 4.81*	06.32	2.23

*Significantly different at $p < 0.05$.**Table 3.** Efficacy of the methanol extract of *I. cylindrica* underground parts on the roundworm, *A. galli* ($n = 6$).

Treatment	Dose (mg/ml)	Survival time (hour) in mean \pm SD	<i>t</i> value	<i>t</i> critical value
Control	0	187.01 \pm 6.77		
Albendazole	20	015.97 \pm 1.99*	28.87	4.30
	10	018.01 \pm 2.73*	56.69	2.23
	5	056.94 \pm 1.76*	45.53	2.23
<i>I. cylindrica</i>	20	102.07 \pm 2.84*	28.34	2.23
	10	140.68 \pm 5.19*	13.40	2.23
	5	183.46 \pm 3.67	01.13	2.31

*Significantly different at $p < 0.05$.

Imperata cylindrica underground parts are traditionally familiar to the Mizo people as anti-infectious agents. They are used as broad-spectrum antimicrobials and anthelmintics for different microbial and helminth infections. Our study shows that the methanol extract is quite effective against pathogenic bacteria, such as *B. subtilis*, *P. aeruginosa*, and *K. pneumonia*. It was most effective on *B. subtilis* on which its efficacy was comparable to that of tetracycline. As secondary metabolites of plants are a good source of antimicrobial agents (Chandra *et al.*, 2017), as such further investigation of this plant is a compelling task to fully explore its pharmacological potential on microbial pathogens of medical importance.

Validating the traditional application, this study also shows that *I. cylindrica* is an effective antiparasitic agent. There are serious concerns in the management of parasitic infections such as drug resistance and adverse effects. These factors raise the need for novel drugs (Clarke *et al.*, 2018). In this context, the plant extract in the present study is remarkable in that it is effective on both the tapeworms and roundworms. This is important because tapeworms and roundworms have contrasting structural and physiological properties (Abongwa *et al.*, 2017).

Tapeworms are soft-bodied helminths without the digestive system so that absorption of nutrients or drugs is direct through the body surface called tegument (Taman and Azab, 2014). Whereas roundworms are hard-bodied helminths covered with tough cuticle and nutrients or drugs are ingested into a digestive tract. The course of drug action is therefore prolonged and requires complex interaction with motor and signaling proteins (Greenberg, 2014). For these reasons, drugs are usually specific for each group

of helminths. The effectiveness of *I. cylindrica* extract against both tapeworms and roundworms as demonstrated in this study is, therefore, an evidence that the plant is a promising source of novel anthelmintic molecules, which exert broad-spectrum activity.

CONCLUSION

GC-MS analysis revealed that the methanol extract of *I. cylindrica* underground part contains important volatile compounds, some of which are known to be pharmacologically bioactive compounds. This is not an exhaustive analysis and the plant is likely to contain more interesting compounds. Validating the medicinal use as antibacterial and antiparasitic agents, the plant extract was effective against three bacteria including Gram-negative bacteria such as *P. aeruginosa* and *K. pneumoniae*, and a Gram-positive bacterium *B. subtilis*. It was an interesting observation that the plant extract was also effective against intestinal parasites such as the tapeworm *R. echinobothrida* and the roundworm *A. galli*.

AUTHORS' CONTRIBUTION STATEMENT

Contribution	P.B. Lalthanpuui	Zarzokimi	K. Lalchandama
Concept and Design	Yes	-	Yes
Experiment	Yes	Yes	-
Data acquisition	Yes	Yes	-
Data analysis	-	-	Yes
Manuscript writing	-	-	Yes
Manuscript editing/review	Yes	Yes	Yes

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CONFLICT OF INTEREST

None declared.

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