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Article *in* Research Journal of Pharmacy and Technology · February 2020

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ISSN 0974-3618 (Print)
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RESEARCH ARTICLE

Analysis of Chemical Constituents and Antiparasitic Activities of the Extracts of *Imperata cylindrica*

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ABSTRACT:

With little known pharmacological potentials, *Imperata cylindrica* is infamous as an invasive grass. But to the Mizo people, the rhizome-root part is a convenient and potent remedy for intestinal helminthiasis. It is an acclaimed effective deworming agent against both tapeworm and roundworm infections, which makes it more interesting for the fact that antiparasitic drugs are usually active against a particular group of helminthic worms. GC-MS analysis of the chloroform extract indicated the presence of 13 volatile compounds, among which *trans*-13-Octadecenoic acid was by far, at relative abundance of 98.4%, the most abundant. There were 17 compounds in the petroleum ether extract with *n*-hexadecanoic acid at relative abundance of 99.7% is the major compound. Biologically active compounds such as 2-methoxy-4-vinylphenol, 6-methylenebicyclo [3.2.0] hept-3-en-2-one, campesterol, and stigmasterol were detected. An *in vitro* test showed that both the extracts were effective against the tapeworm *Raillietina echinobothrida* but only the chloroform at the highest concentration (20 mg/ml) was effective on the roundworm *Ascaridia galli*. These data imply that the plant extracts are a potential source of antiparasitic compounds.

KEYWORDS: *Imperata cylindrica*, GC-MS, parasite, tapeworm, roundworm.

INTRODUCTION:

One of the least investigated but interesting medicinal plants is *Imperata cylindrica* (L.) Räuschel. Considered as an invasive cogon grass, it is a perennial and rhizomatous species that belongs to the family Poaceae. The medicinal properties are well known in different traditional practices in Southeast Asia. The underground part is most commonly used and is applied as antibacterial, anticoagulant (styptic), antipyretic (febrifuge), diuretic, skin softening (emollient), salivating (sialagogue), and soothing (tonic) agent.[1] The roots are particularly effective for the treatment of jaundice, peripheral oedema, and various blood disorders including haematuria (blood urine), haematemesis (blood vomit), and nosebleed (epistaxis).

Received on 03.07.2019 Modified on 10.08.2019
Accepted on 04.09.2019 © RJPT All right reserved
Res. J. Pharm. Technol. 2020; 13(2):653-658
DOI: 10.5958/0974-360X.2020.00125.0

There are only two reports on the bioactive compounds isolated from the leaves, which hare demonstrated to have protective effect on the nervous system,[2] and blood-flow (vasodilative) regulating effects in experimental animals.[3]

The Mizo people of northeast India have several indigenous applications of the plant. In traditional houses, the leaves are the mainstay of thatch roofs for their resilient fibres. The rhizome-root juice is acclaimed as a potent antimicrobial agent. For its ready availability, it is a common therapy for different bacterial infections such as dermal lesion, cholera, diarrhoea and dysentery. It is also used for the treatment of common fungal infections such as ringworms and other skin infections (dermatitis).[4] The most unique medicinal usage is that it is one of the most effective remedies for intestinal worm infection. As an anthelmintic, the underground parts are ground and the juice is directly drunk or are directly chewed to swallow the juice. An interesting impression on this anthelmintic activity is that while most anthelmintic compounds or drugs are specific for a particular group of helminths,

the plant extract is known to be equally effective for both cestodes and nematodes.[5] Therefore, based on the traditional knowledge, it is worthwhile to analyse the chemical components of the plants and evaluate its antiparasitic activity.

MATERIALS AND METHODS:

Plant material:

The whole plants of *Imperata cylindra* were collected from Ngopa, anortheastern village in Mizoram, India, located between 23.8861° latitude north and 93.2119° longitude east. The specimen was authenticated at the Botanical Survey of India (BSI), Shillong, Meghalaya. The voucher herbarium is maintained and catalogued (PUC-I-2018-01) at the herbarium section of Pachhunga University College, Aizawl, India. The aerial parts were cut off and the underground parts were dried in shade at 21-27°C.

Chemicals and drugs:

All chemicals were standard analytical grades procured from HiMedia Laboratories Private Limited, Mumbai, India. Acetonitrile for gas chromatography was a product of Merck Life Science Private Limited, Mumbai, India. Albendazole (Zenlee) was a product of UNI-PEX Pharmaceutical Private Limited, and ceftriaxone (VEGACEF-S) was manufactured by Mak Pharmaceuticals, Sirmaur, Himachal Pradesh, India.

Preparation of plant extracts:

The dried plant parts were ground to course powder using an electric blender. Hot extraction was run continuously in a 5-litre Soxhlet apparatus. Petroleum ether as a highly non-polar solvent and chloroform as a more polar solvent were used for extraction. The extracts were concentrated by removing and recovering the solvents in a vacuum rotary evaporator (Buchi Rotavapor® R-215). The final plant extracts were obtained as semi-solid masses and were stored at 4°C before further use.

GC-MS analysis:

The methanol extract of *I. cylindra* was analysed for chemical identification using gas chromatography-mass spectrometry system (Thermo Scientific TRACE™ 1300 ISQ™ LT). To prepare sample solutions, the plant extracts were dissolved in acetonitrile. A non-polar column TR-5MS (260F142P) was used as a stationary phase and had a dimension of 30 m x 0.25 mm x 0.25 µm with film thickness of 0.25µm. Temperature of the injector port was set at 250°C. The oven temperature was initially set at 70°C for 2 minutes and gradually raised at an increment of 10°C up to 250°C. As a carrier gas, helium was released into the oven chamber at a constant flow rate of 1 mL/min. The samples were injected in a volume of 1µl in split mode and the splitting ratio was maintained at 1:50. The mass spectrometer was run with an ionisation electron energy of 70 eV. Ion source and transfer line temperature were set at 250°C. The total running duration was 60 minutes. The final chromatogram was generated with Thermo Scientific™ Xcalibur™ software. For each sample, compounds were identified on the basis of their retention time, chemical formula and molecular weight from libraries of Wiley Registry™ and National Institute of Standards and Technology database.

Anthelmintic test:

In vitro anthelmintic activity was studied on two intestinal parasites, namely a tapeworm *Raillietina echinobothrida* and a roundworm *Ascaridia galli.* The helminth parasites were recovered and collected from the intestines of freshly sacrificed local fowls, *Gallus gallus domesticus.* An hour before the experimental assay, the plants extracts were made in solutions of varying concentrations such as 0.5, 1, 2, 5, 10 and 20 mg/ml in culture plates by dissolving them in 0.9% neutral phosphate-buffered saline (PBS) supplemented with 1% dimethylsulfoxide (DMSO). Corresponding concentrations of albendazole were also prepared as standard references. Control media consisted only of PBS with DMSO. Batches of 3 worms were selected for each test, and each test was further performed in triplicates. They were incubated in a biological incubator maintained at a constant temperature of 37±1°C.

Anthelmintic efficacy was assessed in terms of survival in the culture media. Death was defined as no further motor activity even after stimulation by dipping in lukewarm PBS at maintained at 45°C. The durations of survival were recorded, and data were generated as statistical means ± standard deviation. Significance of the anthelmintic activity was determined using Student's *t*-test, and the level of significance was considered when *p*value was <0.05.

RESULTS:

Chemical analysis using GC-MS:

GC-MS chromatogram of the petroleum ether extract of *I. cylindrica* obtained is shown in Figure 1. Table 1 shows the list of compounds detected from the chromatogram. From it, the presence of 13 compounds was confirmed. *trans*-13-Octadecenoic acid is by the most abundant with relative abundance of 98.4%, followed by (Z)-18-octadec-9-enolide at 70.2%. *n*-Hexadecanoic acid (68.2%) and octadecanoic acid (43.7%) were also present in fair amount.

From the chromatogram of the chloroform extract (Figure 2), 17 compounds were identified as shown in Table 2.

Table 1:GC-MS analysis of the petroleum ether extract of *I. cylindrica*.

Sl. No.	Retention Time	Compound	Formula	Molecular weight	Relative abundance (%)
1.	15.32	2,5-Dihydro-1-nitroso-1H-pyrrole	$C_4H_6N_2O$	98	9.2
2.	20.05	13-Heptadecyn-1-ol	$C_{17}H_{32}O$	252	8.9
3.	28.68	2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	$C_{16}H_{30}O_4$	286	37.4
4.	35.64	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	23.4
5.	36.41	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	68.2
6.	39.66	(Z)-18-Octadec-9-enolide	$C_{18}H_{32}O_2$	280	70.2
7.	39.72	*trans*-13-Octadecenoic acid	$C_{18}H_{34}O_2$	294	98.4
8.	40.12	Octadecanoic acid	$C_{18}H_{36}O_2$	284	43.7
9.	43.18	Octadecanal,2-bromo	$C_{18}H_{35}BrO$	346	16.7
10.	44.25	Octadecanoic acid, butyl ester	$C_{22}H_{44}O_2$	340	18.25
11.	46.47	Diisooctyl phthalate	$C_{24}H_{38}O_4$	390	22.7
12.	49.39	17α,21β-28,30-bisnorhopane	$C_{28}H_{48}$	384	16.2
13.	52.86	9,19-Cycloergost-24(28)-en-3-ol,4,14-dimethyl, acetate, (3β,4α,5α)	$C_{32}H_{52}O_2$	468	17.4

Table 2:GC-MS analysis of the chloroform extract of *I. cylindrica*.

Sl. No.	Retention time	Compound	Formula	Molecular weight	Relative abundance (%)
1.	10.37	Benzenepropanoic acid, 3,5-bis (1,1-dimethylethyl)-4-hydroxy, octadecyl ester	$C_{36}H_{62}O_3$	530	7.1
2.	17.46	13-Heptadecyn-1-ol	$C_{17}H_{32}O$	252	5.7
3.	20.04	Decanoic acid, 3-hydroxy	$C_{12}H_{24}O_3$	216	5.9
4.	26.64	Phenol,2,4-bis(1,1-dimethylethyl)	$C_{14}H_{22}O$	206	76.5
5.	28.51	Hexadecen-1-ol, trans-9	$C_{16}H_{32}O$	240	64.2
6.	32.93	E-15-Heptadecenal	$C_{17}H_{32}O$	252	66.5
7.	36.59	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	99.7
8.	36.95	1-Heneicosyl formate	$C_{22}H_{44}O_2$	340	48.7
9.	39.71	(Z)-18-octadec-9-enolide	$C_{18}H_{32}O_2$	280	76.3
10.	39.79	*cis*-Vaccenic acid	$C_{18}H_{34}O_2$	282	71.7
11.	40.19	Octadecanoic acid	$C_{18}H_{36}O_2$	284	65.7
12.	40.61	Heptacos-1-ene	$C_{27}H_{54}$	378	24.1
13.	43.48	4(4-Chlorphenyl)-3-morpholinopyrrol-2-carboxylic acid, methyl ester	$C_{16}H_{17}C_1N_2O_3$	320	11.5
14.	46.46	Diisooctyl phthalate	$C_{24}H_{38}O_4$	390	49.4
15.	48.15	17α,21β-28,30-Bisnorhopane	$C_{28}H_{48}$	384	12.2
16.	50.26	Campesterol	$C_{28}H_{48}O$	400	11.5
17.	51.56	Stigmasterol	$C_{29}H_{48}O$	412	16.7

Table 3. Efficacy of the chloroform and petroleum ether extracts of *I. cylindrica* on the tapeworm, *Raillietina echinobothrida* (n = 6).

Treatment	Dose (mg/ml)	Survival time (hour) in mean ± SD	t value	t critical value
Control	0	74.03 ± 1.89	NA	NA
Albendazole	5	14.99 ± 0.43*	74.53	2.23
	10	12.07 ± 0.49*	77.66	2.23
	20	08.99 ± 0.45*	81.85	2.23
I. cylindrica chloroform extract	5	59.10 ± 4.17*	06.32	2.23
	10	47.82 ± 5.83*	12.06	2.23
	20	26.03 ± 6.23*	28.99	2.23
*I. cylindrica*petroleum ether extract	5	69.76 ± 5.06*	06.32	2.23
	10	59.10 ± 4.17*	12.06	2.23
	20	48.69 ± 3.61*	28.99	2.23

*Significantly different at $p < 0.05$; NA = not applicable.

Table 4. Efficacy of the chloroform and petroleum ether extracts of *I. cylindrica* on the roundworm, *Ascaridia galli* (n = 6).

Treatment	Dose (mg/ml)	Survival time (hour) in mean ± SD	t value	t critical value
Control	0	187.01 ± 6.77	NA	NA
Albendazole	5	056.94 ± 1.76*	45.53	2.23
	10	018.01 ± 2.73*	56.69	2.23
	20	015.97 ± 1.99*	28.87	4.30
I. cylindrica chloroform extract	5	182.43 ± 5.59	01.74	2.23
	10	180.95 ± 5.19	01.20	2.23
	20	177.56 ± 5.09*	02.74	2.31
I. cylindrica petroleum ether extract	5	183.55 ± 5.82	00.97	2.23
	10	185.82 ± 4.23	00.37	2.23
	20	186.20 ± 4.01	00.25	2.23

*Significantly different at $p < 0.05$; NA = not applicable.

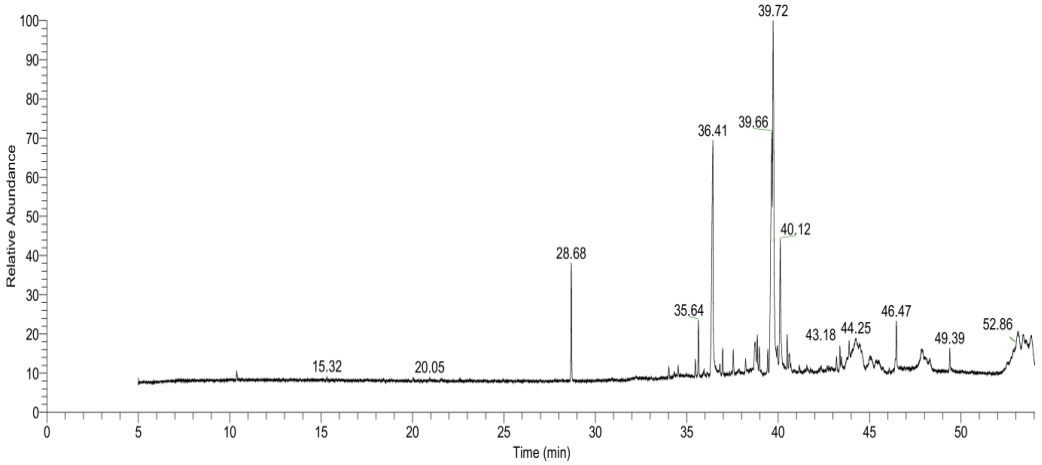

Figure 1. GC-MS chromatogram of the petroleum ether extract of *Imperata cylindrica.*

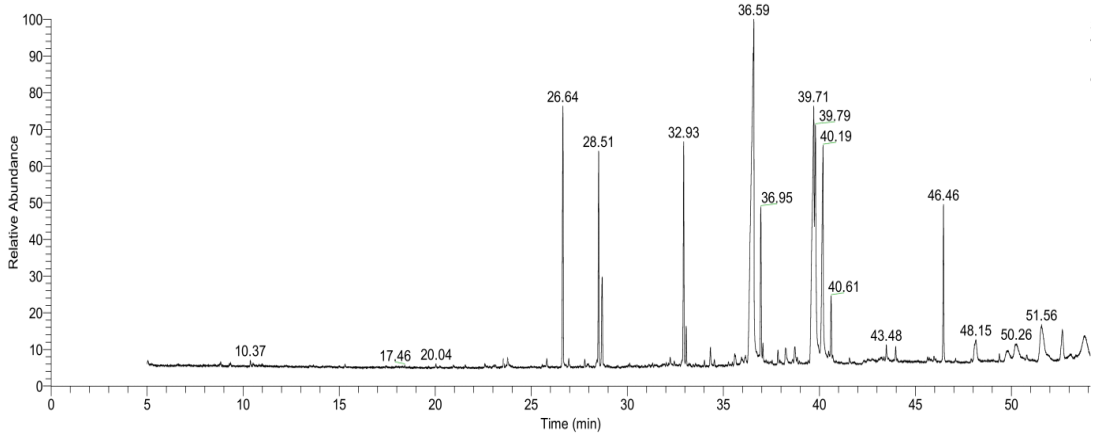

Figure 2. GC-MS chromatogram of the chloroform extract of *Imperata cylindrica.*

The most abundant was *n*-hexadecanoic acid at relative abundance of 99.7%. Phenol,2,4-bis(1,1-dimethylethyl) (76.5%), hexadecen-1-ol, trans-9 (64.2%), E-15-heptadecenal (66.5%), (Z)-18-octadec-9-enolide (76.3%), *cis*-vaccenic acid (71.7%), and octadecanoic acid (65.7%) were other major compounds. Detected in less quantities, but biologically important compounds included campesterol (11.5%) and stigmasterol (16.7).

Antiparasitic activity:

The antiparasitic efficacy of albendazole, chloroform and petroleum ether extracts of *I. cylindrica* on the tapeworm, *Raillietina echinobothrida*, is given in Table 3. Worms in control experiment lived for 74.03 ± 1.89 hours. Significant concentration-dependent effects were seen in all the tests. Albendazole was most effective and killed all the worms in 14.99 ± 0.43, 12.07 ± 0.49, and 08.99 ± 0.45 hours at the concentrations of 5, 10, and 20 mg/ml respectively. The chloroform extract of *I. cylindrica* was more potent than the petroleum ether extract, taking 59.10 ± 4.17, 47.82 ± 5.83, and 26.03 ± 6.23 hours to kill all the parasites; while the petroleum ether extract required 69.76 ± 5.06, 59.10 ± 4.17, and 48.69 ± 3.61 hours at similar concentrations.

Albendazole was again highly effective on the roundworm, *Ascaridia galli*, as shown in Table 2. Roundworms survived relatively longer, up to 187.01 ± 6.77 hours. Albendazole took 056.94 ± 1.76, 018.01 ± 2.73, and 015.97 ± 1.99 hours to kill all the parasites at the concentrations of 5, 10, and 20mg/ml respectively. The plant extracts were not significantly effective, except for 20mg/ml of the chloroform extract which killed the worms at 177.56 ± 5.09 hours.

DISCUSSION:

Many compounds that have been identified from the underground parts of *I. cylindrica* are already known to have considerable biological effects. For example, 2-methoxy-4-vinylphenol isolated from isolated from the needles of pine (*Pinus* species) is experimentally established to have anticarcinogenic activity by blocking the hyper-phosphorylation of retinoblastoma protein *in vitro*.[6] It additionally enhances antiinflammatory response suppression of NF-κB and MAPK activation, and acetylation of histone H3.[7] Another compound, 6-methylenebicyclo [3.2.0] hept-3-en-2-one reported from *Allium tuberosum* is an important immune molecule for the plant against parasitic infection such as root-knot

nematode, *Meloidogyne* species.[8] Avocado (*Perseaamericana*) contains phenol,2,4-bis (1,1-dimethylethyl) that act as antifungal compound against *Aspergillus* and *Phytophthora cinnamomi*.[9]

Campesterol, known from a wide variety of plants, is an established phytocompound with blood cholesterol-lowering and anticarcinogenic properties. The sterol compound isolated from *Chrysanthemum coronarium* was shown to inhibit fibroblast growth factor (bFGF) and tube formation of human umbilical vein endothelial cells, thereby implicating it in the prevention of blood cancers.[10] In experimental mice, rich phytosterol diet increased circulating β-sitosterol and campesterol, which then inhibit cancer cells (MDA-MB-231).[11] In human studies, there have been contradicting reports. Some studies have shown that high phytoestrogen diet containing β-sitosterol, campesterol, and stigmasterol decreased the risk of gastric cancer,[12] and lung cancer.[13] But a study reported that campesterol and stigmasterol increased the risk of colon cancer.[14] Nonetheless, the general conception is that these phytosterols are beneficial for cardiovascular disease and cancer ricks.[15] Stigmasterol is additionally associated with prevention of cartilage degradation (osteoarthritis) by inhibiting proteins involved in the functions of chondrocytes.[16] It was further shown to reduce serum thyroid hormones, namely triiodothyronine (T_3) and thyroxin (T_4), as well the activity of hepatic glucose-6-phophatase, thereby reducing blood glucose level.[17]

Corroborating the traditional application, our data reveal that the underground parts of *I. cylindrica* can be effective antiparasitic agents. There are alarming situations in both human and veterinary medications specifically in the management of parasitic infections because of drug resistance and adverse effects of available drugs. Hence, a relentless search for novel drugs is one major scientific focus.[18] The effectiveness of *I. cylindrica* extracts as shown here is a promising lead source. They are exceptionally active at all concentrations tested against tapeworms. Even though only the chloroform extract at high concentration (20 mg/ml) was active, it was indicative that antiparasitic compounds are present perhaps in small quantities. These contrasting effects are to be expected because tapeworms and roundworms as two complete distinct classes have utter differences in structural and physiological properties.[19] As soft-bodied helminths without digestive system, absorption of nutrients or drugs is direct through the body surface called tegument in tapeworms; meaning that drugs act faster.[20] Whereas roundworms are hard-bodied helminths covered with tough cuticle so that nutrients or drugs are absorbed only in the digestive tract; thereby prolonging the course of drug action.[21] For these reasons, antiparasitic drugs are usually specific for each group of parasites, and it is possible to infer that the plant extracts contain one or more compounds that could be a useful lead molecule as a broad-spectrum antiparasitic agent.

CONCLUSION:

Chemical identification using GC-MS shows that the chloroform and petroleum ether extracts of *I. cylindrica* underground part contain several pharmacologically important volatile compounds, many of which are already known to be potential pharmaceutical lead molecules. As restricted to the volatile components only, this study is not exhaustive and further analysis is likely to yield more interesting compounds. In conjunction with the traditional usage among the Mizo people as deworming agents for intestinal infections, the plant extracts were effective against parasitic worms such as the tapeworm *Raillietina echinobothrida* and the roundworm *Ascaridia galli.* Both the plant extracts were highly effective on the tapeworms, but only the chloroform extract at high concentration was effective on the roundworms.

ACKNOWLEDGEMENT:

The research is supported by Science and Engineering Research Board (SERB), Government of India, through research project no. EMR/2016/004053 of 23/03/2017. PBL is a Senior Research Fellow under the project. GC-MS facility was provided by the Director, Assistant Director (Chemistry) and Junior Scientific Officer (Chemistry) at the Directorate of Forensic Science Laboratory, Government of Mizoram, Aizawl, India.

CONFLICT OF INTEREST:

None declared.

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