Heavy metal and HPTLC fingerprint analysis of Siddha drug, *Pun podi* prepared from *Datura metel*

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Abstract

Pun podi, a potent tropical combination used in Siddha medicine, contains sulfur and *Datura metel* fruit. Despite its importance, research into the various compositions of *Pun podi* due to diverse preparation methods and seasonal variations remains lacking. To achieve the above objective, the present study was conducted. The *Pun podi* was prepared in three ways by using *Datura metel* fruit, collected in three different seasons. The different methods used to prepare three *Pun podi* and named that different *Pun podi* as *Pun podi* 1, 2, and 3. Heavy metal analysis was performed by using Bruker S8-Tiger WD-XRF analyzer. High-Performance Thin Layer Chromatography was used to analyze fingerprints on three different samples. The data was then analyzed using a standard statistical approach SPSS to identify significant differences between the samples throughout different seasons. SO_3, K_2O, P_2O_5, Ca, Na_2O, SiO_2 Cl, MoO_3 showed significant differences ($p <0.05$) between the *Pun podi* prepared by different methods while MgO, PbO, TiO_2, Al_2O_3, and Fe_2O_3 did not show significant differences ($p >0.05$) among *Pun podi*. Statistically significant disparities were observed in the oxide form of microelements present in *Pun podi*, derived from *Datura metel* fruit collected during different seasons ($p < 0.05$). Specifically, other microelements except Fe_2O_3, PbO, and TiO_2 in *Pun podi* showed notable discrepancies ($p < 0.05$) linked to seasonal variations. Furthermore, Mg, Ca, Si, Pb, Ti, Mo, and Fe demonstrated insignificant differences among *Pun podi* prepared through various methods ($p > 0.05$). However, excluding these microelements, significant distinctions were noted across other elements in *Pun podi* derived from different preparation methods ($p < 0.05$). Beside P, Fe, Mo and other microelements showed significant differences with respect to seasons ($p < 0.05$). *Pun podi* 2 notably contained a higher concentration of both oxidized and elemental microelements compared to other *Pun podi* variants. Evidently, both seasonal factors and preparation methods exerted discernible influences on the microelement composition of *Pun podi*. HPTLC results showed, 04 spots in *Pun podi* 2, 2 spots in *Pun podi* 1, and one spot in *Pun podi* 3, at 254 nm. The values obtained from these tests were less than one.

Keywords: *Datura metel*, Heavy metals, Microelements, *Pun podi*

Introduction

According to WHO estimates, at least 80% of the world's population, mostly in developing nations, still relies on herbal remedies for their basic medical needs. Traditional medicine is used due to its availability, affordability and people's religious beliefs which firmly ingrained. Further, traditional knowledge is an important resource for any nation since it helps the country to advance and transform its society[1]. Nature has given us several plants on this planet that are traditionally utilized as medicines by many different ethnic groups. The plants have long

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been used to treat a variety of wounds and disorders affecting the tissues. It seems essential to conduct more scientific research to confirm the therapeutic uses of medicinal plants for treating wounds and the compositions that influence wound healing[2]. In Sri Lanka, three main indigenous medical systems are practiced; Ayurveda, Siddha, and Unani. Plants are fundamental to all three of the indigenous systems. Siddha medicine, whose origins go back to BC 10000–BC 4000, is the most widely practiced medicinal system worldwide[3].

Datura metel (*D. metel*) is a wild plant that thrives in hotter climates and is used in gardens all over the world for both its medicinal and aesthetic qualities. *D. metel* is generally an annual herbaceous plant. It expands to a height of over twelve feet and sprouts many branches[4]. *D. metel* is currently widespread throughout Southeast Asia and probably originated in Northern India. In tropical areas of the world, it is cultivated as a source of the alkaloid, scopolamine[5].

In Siddha treatment, the *Pun podi*, which contains unripe *D. metal* fruit and sulfur as components, is applied externally. It is applied topically on ulcers, scabies, and itching. In addition to their antiseptic properties, all of the components are said to offer analgesic and anti-microbial properties. *Pun podi* is a Siddha medicinal powder that has historically been applied topically to cure ulcers, and until 1995 there was no scientific evidence to support its usage in wound therapy that has been reported.

Materials and Methods

Collection of the materials

D. metel fruits were collected from seven different places in Sri Lanka and taxonomically identified by the National Herbarium Center, Peradeniya. The fruits were thoroughly washed with running water. The *Pun podi* was prepared after it had been dried and stored in a shady place. The same procedure was carried out three times to create three *Pun podi* spaced three months apart.

Preparation of medicine

Pun podi 1, 2 and 3 were prepared according to the procedure described by Piratheepkumar et al.[6]

Extraction of Pun podi

Cold water extraction

The *Pun podi,* 10 mg, was crushed with 10 ml of distilled water using a motor and pestle and centrifuged for 10 minutes at 10,000 rpm. The supernatant was separated carefully.

Hot water extraction

Pun podi, 10 mg, and 10 ml of distilled water were crushed well using a motor and pestle and placed in a boiling water bath (100°C) for 5 minutes. Then it was cooled and centrifuged for 10 minutes at 10,000 rpm. The supernatant was separated carefully.

HPTLC fingerprint

Preparation of Test solution: About 2.5 g of *Pun podi*, prepared by different methods with methanol for 24 hours, was macerated. It was filtered, evaporated and the dried residue was dissolved in methanol and used for TLC analysis.

Procedure: Silica Gel 60 F_{254} were used as the stationary phase while using the solvent system of Toluene: Ethyl acetate: Diethylamine in a 7:2:1 ratio, as the mobile phase. 20µl of prepared test solutions (T1, T2, T3) were applied on a pre-coated silica gel 60 F_{254} HPTLC plate (E. Merck) of uniform thickness of 0.2mm using Linomat 05 sample applicator. Developed the plate in the solvent system to a distance of 8cm. The plates were observed under UV light at 254nm using CAMAG REPROSTAR-3.

X-Ray Fluorescence (XRF) analysis

X-Ray Fluorescence (XRF) analysis technique was performed based on Mokhena et al., (2016) [7] by using Bruker S8-Tiger WD-XRF analyzer equipped with a Rh-anticathode X-ray tube, five analyzing crystals, different collimators and filters, a flow proportional counter for light elements (Na to V) and a scintillation counter for heavy elements (Cr to Pb).

Results

Table 1 shows the mean value of oxide form of microelements of *Pun podi* prepared by using *D. metel* fruit collected in different seasons. SO_3 was found in high amount among all oxide forms of microelements of the all *Pun podis*. However, it was high in *Pun podi 1* prepared by using *D. metel* collected in May compared to other *Pun podis* prepared in different time periods. The large number of oxide form of microelements found in *Pun podi 2* among the *Pun podis* prepared with different methods. MoO_3 was not found in all *Pun podi 1* samples prepared in three different periods while TiO_2 was not found in *Pun podi 3*. PbO was found small amount in *Pun podi* 1 prepared by using *D. metel* collected in May only. Other *Pun podi 1* samples prepared in January and September were not contained PbO.

Table 2 shows the differences of oxide forms of microelements of the *Pun podi* prepared by different methods. Among the oxide forms of microelements, SO_3 was found high in all *Pun podi* and it was found high in *Pun podi 1* compared to other *Pun podis* prepared by different methods. SO_3 and K_2O were found in high amount among the all-oxide forms of microelements and it was high in *Pun podi 2* compared to other *Pun podi*.

Table 3 shows the presence of elemental forms of microelements of *Pun podi* prepared by using *D. metel* fruit collected in different seasons at three months' interval.

Elemental sulfur was found in high amount among the microelements of all *Pun podis*. However, it was high in *Pun podi 1* prepared by using *D. metel* collected in January compared to other *Pun podis* prepared in different periods. The large number of microelements found in *Pun podi 2* among the *Pun podis* prepared with different methods by using *D. metel* collected in different seasons. MoO_3 was not found in *Pun podi 1* & 2 prepared in three different periods while TiO_2 and PbO were not found in *Pun podi 3* and 1 respectively.

Referring to table 4, the elemental sulfur was found in high amount in *Pun podi* 1 among the *Pun podis* prepared in different methods. Except elemental sulfur, K was observerd in high amount in *Pun podi 2*.

Table 1: The mean differences of oxide form of microelements of *Pun podi* prepared by using *D. metel* fruit collected in different seasons

Sample	Season		SO_3	K_2O	P_2O_5	MgO	CaO	Na_2O_3	SiO_2	Cl	Al_2O_3	Fe_2O_3	MoO_3	PbO	TiO_2
Pun podi 1	***Jan***	Mean	87.80	1.25	0.20	1.63	1.27	1.24	3.10	1.36	0.56	0.03	0	0	0.03
		SD	1.94	0.06	0.02	0.10	0.03	0.09	0.12	0.07	0.07	0.01	0	0	0.02
	May	Mean	90.81	1.35	0.35	1.63	1.37	1.18	3.58	1.92	0.85	0.04	0	0.01	0.01
		SD	1.62	0.05	0.03	0.10	0.04	0.04	0.14	0.05	0.05	0.02	0	0.01	0.01
	Sep	Mean	90.63	1.26	0.25	1.91	1.20	1.28	3.27	1.32	0.70	0.04	0	0	0.02
		SD	0.61	0.05	0.02	0.10	0.07	0.04	0.06	0.02	0.04	0.03	0	0	0.01
Pun podi 2	***Jan***	Mean	46.53	37.18	6.14	4.35	3.01	0.55	1.13	4.72	0.40	0.05	0.01	0.01	0.47
		SD	2.84	2.07	0.06	0.40	0.41	0.10	0.05	0.08	0.01	0.01	0.01	0.01	0.15
	May	Mean	47.64	40.38	6.09	4.44	2.94	0.61	1.05	4.92	0.52	0.05	0.02	0.01	0.40
		SD	1.13	0.95	0.09	0.41	0.09	0.09	0.04	0.10	0.03	0.02	0.01	0.01	0.10
	Sep	Mean	45.76	33.64	6.53	4.15	2.89	0.49	1.11	4.24	0.33	0.04	0.02	0.02	0.37
		SD	2.21	1.82	0.19	0.06	0.19	0.04	0.04	0.33	0.03	0.01	0.01	0.01	0.06
Pun podi 3	***Jan***	Mean	70.95	23.04	2.99	1.67	1.58	0.66	0.49	0.27	0.17	0.03	0.02	0.02	0
		SD	0.38	1.58	0.14	0.21	0.12	0.06	0.05	0.06	0.12	0.02	0.01	0.01	0
	May	Mean	73.12	21.86	2.49	1.78	1.55	0.71	0.58	0.37	0.27	0.02	0.02	0.02	0
		SD	3.35	0.90	0.14	0.36	0.17	0.10	0.04	0.06	0.12	0.01	0.01	0.01	0
	Sep	Mean	72.21	25.38	2.90	2.09	1.51	0.63	0.70	0.37	0.30	0.04	0.02	0.01	0
		SD	5.06	0.66	0.10	0.07	0.08	0.05	0.04	0.25	0.26	0.03	0.01	0.01	0

Table 2: The differences of oxide form of microelements between the *Pun podi* prepared by different methods

Method		SO_3	K_2O	P_2O_5	MgO	CaO	Na_2O	SiO_2	Cl	Al_2O_3	Fe_2O_3	MoO_3	PbO	TiO_2
1	Mean	89.74	1.29	0.27	1.72	1.28	1.23	3.32	1.53	0.70	0.04	0.00	0.00	0.02
	SD	1.96	0.06	0.07	0.16	0.09	0.07	0.23	0.29	0.13	0.02	0.00	0.00	0.01
2	Mean	46.64	37.07	6.25	4.31	2.94	0.55	1.10	4.63	0.42	0.04	0.02	0.01	0.41
	SD	2.06	3.27	0.23	0.32	0.24	0.09	0.05	0.35	0.09	0.01	0.01	0.01	0.11
3	Mean	72.09	23.42	2.79	1.85	1.55	0.66	0.59	0.33	0.24	0.03	0.02	0.02	0.00
	SD	3.18	1.83	0.26	0.28	0.12	0.07	0.10	0.14	0.17	0.02	0.01	0.01	0.00

Table 3: The mean differences of elemental form of microelements of *Pun podi* prepared by using *D. metel* collected in different seasons

	Season		S	K	P	Mg	Ca	Na	Si	C	Al	Fe	Mo	Pb	Ti
Pun podi 1	Jan	Mean	92.1	1.05	0.16	1	0.96	0.83	1.56	1.31	0.3	0.01	0	0	0.02
		SD	0.9	0.03	0.11	0.07	0.04	0.07	0.05	0.04	0.01	0.00	0	0	0
	May	Mean	89.33	1.00	0.63	1.03	1.04	1.00	1.74	1.23	0.24	0.04	0	0	0.02
		SD	2.95	0.06	0.20	0.03	0.06	0.08	0.06	0.10	0.02	0.02	0	0	0.01
	Sept	Mean	90.73	1.05	0.13	1.05	1.13	0.91	1.49	1.15	0.31	0.01	0	0	0.03
		SD	2.36	0.03	0.05	0.05	0.06	0.02	0.07	0.05	0.02	0.00	0	0	0.02
Pun podi 2	Jan	Mean	59.18	30.9	2.76	2.41	1.92	0.31	0.37	4.63	0.17	0.03	0	0.01	0.02
		SD	2.29	0.38	0.12	0.04	0.04	0.02	0.28	0.28	0.05	0.01	0	0.00	0.01
	May	Mean	52.08	36.5	3.01	2.81	2.06	0.31	0.47	5.23	0.22	0.03	0	0.03	0.02
		SD	2.81	3.96	0.10	0.1	0.06	0.04	0.07	0.41	0.03	0.01	0	0.01	0
	Sept	Mean	63.08	30.4	2.66	2.43	1.80	0.42	0.43	5.32	0.23	0.01	0	0.02	0.01
		SD	3.05	0.43	0.06	0.06	0.04	0.04	0.05	0.30	0.06	0.00	0	0.00	0.00
Pun podi 3	Jan	Mean	77.16	17.7	1.25	0.96	1.13	0.48	0.24	0.26	0.05	0.01	0.01	0.01	0
		SD	0.31	0.73	0.02	0.03	0.04	0.03	0.05	0.04	0.01	0.00	0.00	0.00	0
	May	Mean	80.10	20.0	1.36	1.06	0.99	0.51	0.39	0.21	0.08	0.01	0.07	0.02	0
		SD	0.85	0.94	0.06	0.07	0.02	0.06	0.01	0.03	0.01	0.00	0.00	0.01	0
	Sept	Mean	78.89	19.4	1.27	1.02	1.1	0.53	0.4	0.32	0.03	0.03	0.01	0.01	0
		SD	2.34	1.41	0.06	0.11	0.03	0.06	0.04	0.07	0.01	0.01	0.00	0.00	0

Table 4: The differences of elemental form of microelements of *Pun podi* prepared by different methods

Method		S	K	P	Mg	Ca	Na	Si	C	Al	Fe	Mo	Pb	Ti
1	**Mean**	90.72	1.04	0.31	1.03	1.05	0.92	1.60	1.23	0.28	0.02	0.00	0.00	0.02
	SD	2.28	0.05	0.27	0.06	0.09	0.09	0.13	0.09	0.04	0.02	0.00	0.00	0.01
2	**Mean**	58.12	32.63	2.81	2.55	1.93	0.35	0.43	5.07	0.21	0.03	0.00	0.02	0.02
	SD	5.38	3.57	0.18	0.20	0.12	0.07	0.15	0.44	0.05	0.01	0.00	0.00	0.01
3	**Mean**	78.72	19.09	1.29	1.02	1.07	0.51	0.35	0.27	0.06	0.02	0.04	0.02	0.00
	SD	1.79	1.39	0.07	0.08	0.07	0.05	0.08	0.07	0.03	0.01	0.03	0.00	0.00

HPTLC fingerprinting profile

HPTLC results showed (Figure 1 and 2) (Table 5), 04 spots in *Pun podi* 2, 2 spots in *Pun podi* 1, and one spot in *Pun podi* 3, at 254 nm.

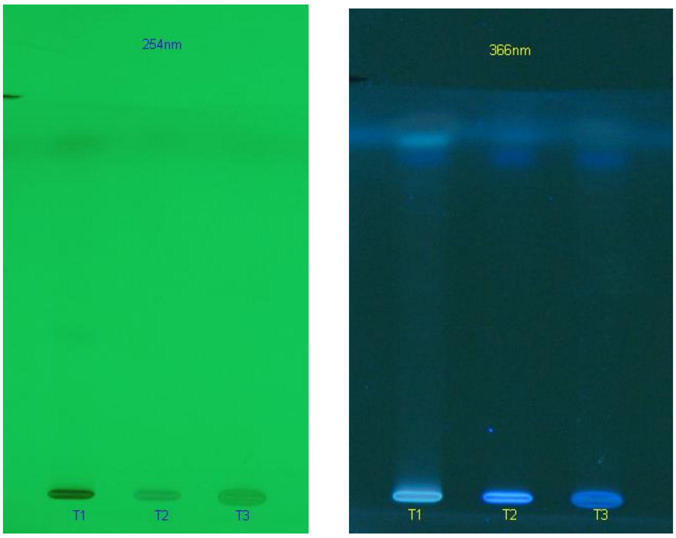

Figure 1: HPTLC plate of *Pun podi* prepared by different methods

***Pun podi* 1** ***Pun podi* 2** ***Pun podi* 3**

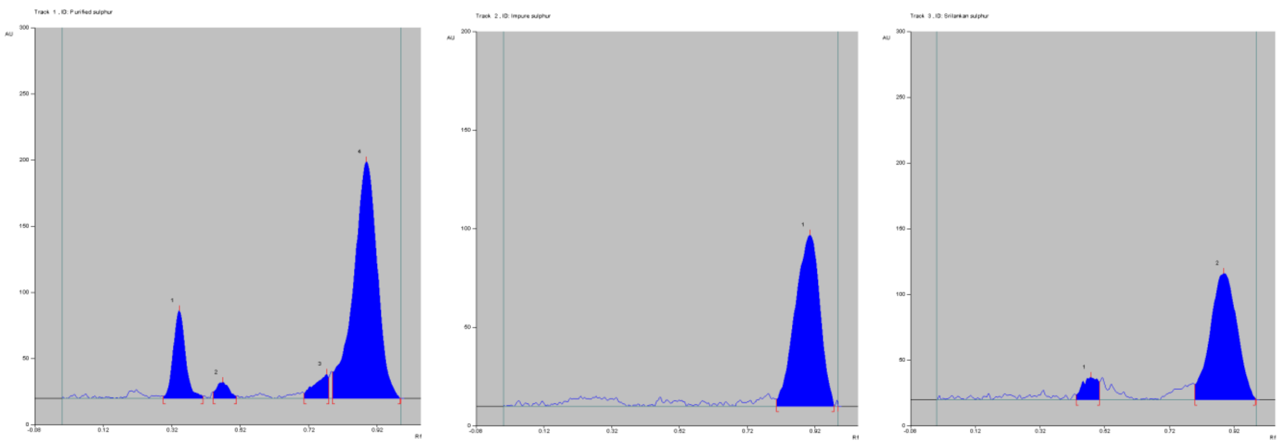

Figure 2: HPTLC fingerprinting of *Pun podi*

Table 5: Peak list of Toluene: Ethyl acetate: Diethylamine extract of *Pun podi* at UV =254 nm

Sample	Peak	Start Rf	Start Height	Max rf	Max Height	Height %	End Rf	End Height	Area	Area %
Pun podi 1	1	0.43	3.8	0.48	16.9	14.96	0.50	13.7	596.4	9.44
	2	0.80	11.6	0.89	96.0	85.04	0.99	1.3	5718.9	90.56
Pun podi 2	1	0.30	1.7	0.34	66.2	24.02	0.41	1.6	1672.2	14.63
	2	0.44	5.1	0.47	12.2	4.42	0.51	1.7	326.0	2.85
	3	0.71	3.8	0.77	18.3	6.65	0.78	15.4	525.3	4.60
	4	0.79	19.9	0.89	179.0	64.91	0.99	0.1	8906.3	77.92
Pun podi 3	1	0.81	3.5	0.91	86.8	100.00	0.98	0.5	4546.0	100

Discussion

The season had an impact on the amounts of P, Na, and Fe accumulated in the plants[8]. In winter (December to February) more P and Na have accumulated, while the summer-autumn (March–May, and October–November) and winter periods showing the highest amounts of Fe accumulation. Season did not affect the accumulation of N, K, Ca, Mg, Zn, and Cu according to a recent study[8]. According to the present study, the *Pun podi* showed high Na in May (summer) (1.0% ± 0.08%) compared to other seasons (Table 3). It showed a significant difference ($p < 0.05$) between *Pun podi* prepared by *D. metel* collected in different seasons (Table 7). However, the accumulation of P and Fe was insignificant in *Pun podi* prepared in different seasons (Table 9). Accumulation of K, Ca, and Mg was significant ($p < 0.05$) in the *Pun podi* prepared by using *D. metel* collected in different seasons (Table 9).

In wound healing, Iron is known for its oxidative role in hemostasis, in which ferrous iron is released from hemoglobin which acts to promote blood clotting[9] and it's an important component of many antioxidant processes[10,11]. Certain enzymes need iron to get activate and stimulate angiogenesis during wound healing[12]. Iron is a component or activator of enzymes which important in several steps of the healing process. It is also important in anti-inflammatory therapy[13]. The presence of Fe, in both elemental and oxide forms, in *Pun podi* was observed prepared by using *D. metel* collected in different seasons (Table 1 and 3). There were insignificant differences ($p > 0.05$) in the amount of Fe present in both elemental and oxide forms between the *Pun podi* prepared by different methods (Table 6 and 7). It was implied that there were no between the presence of iron and the methods of preparation of *Pun podi*. However, significant changes ($p < 0.05$) were observed in the presence of Fe in both elemental and oxide forms among the *Pun podi* prepared in three different seasons (Table 8 and 9).

Soft tissue and magnesium ions (Mg^{2+}) are tightly related to wound healing. According to Yang et al., (2023) magnesium ions can also encourage angiogenesis[14]. According to Razzaghi et al., (2018)[15] 12-week treatment with Mg^{2+}, significantly improved wound healing of the individuals with diabetic foot ulcers. It has anti-inflammatory action also which is essential in wound healing[15] . In the present study, all *Pun podi* prepared by different methods showed the presence of Mg in both elemental and oxide forms (Table 2 and 4). Above studies emphasize the importance of the literature from where the drug has been selected to determine the effectiveness for wound healing. Coger et al., (2019)[16] stated that the concentration of iron and magnesium should be

Table 6: Statistical difference of oxide form of microelements between the *Pun podi* prepared in different methods

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	SD	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	**$1SO_3$ - $2SO_3$**	43.100	1.8057	1.0425	38.61422	47.58585	41.340	2	.001
Pair 2	**$1SO_3$ - $3SO_3$**	17.611	.73150	.42233	15.79400	19.42827	41.700	2	.001
Pair 3	**$2SO_3$ - $3SO_3$**	-25.48	1.0767	.62165	-28.1636	-22.81415	-41.002	2	.001
Pair 4	**$2K_2O$ - $2K_2O$**	-35.77	3.3339	1.9248	-44.0598	-27.49572	-18.587	2	.003
Pair 5	**$1K_2O$ - $3K_2O$**	-22.13	1.8270	1.0548	-26.6752	-17.59813	-20.986	2	.002
Pair 6	**$2K_2O$ - $3K_2O$**	13.641	5.1512	2.9740	.84478	26.43742	4.587	2	.044
Pair 7	**$1P_2O_5$ - $2P_2O_5$**	-5.989	.26570	.15340	-6.64971	-5.32962	-39.045	2	.001
Pair 8	**$1P_2O_5$ - $3P_2O_5$**	-2.526	.33970	.19613	-3.37010	-1.68236	-12.881	2	.006
Pair 9	**$2P_2O_5$ - $3P_2O_5$**	3.4634	.26871	.15514	2.79592	4.13094	22.325	2	.002
Pair 10	**1MgO - 2MgO**	-2.592	.30574	.17652	-3.35169	-1.83271	-14.685	2	.005
Pair 11	**1MgO - 3MgO**	-.1233	.07621	.04400	-.31264	.06597	-2.803	2	.107
Pair 12	**2MgO - 3MgO**	2.4688	.35142	.20289	1.59588	3.34185	12.168	2	.007
Pair 13	**1CaO - 2CaO**	-1.664	.08754	.05054	-1.88170	-1.44677	-32.928	2	.001
Pair 14	**1CaO - 3CaO**	-.2631	.07518	.04340	-.44988	-.07639	-6.063	2	.026
Pair 15	**2CaO - 3CaO**	1.4011	.03145	.01816	1.32296	1.47924	77.151	2	.000
Pair 16	**$1Na_2O$ - $2Na_2O$**	.68553	.11007	.06355	.41211	.95896	10.788	2	.008
Pair 17	**$1Na_2O$ - $3Na_2O$**	.56830	.08799	.05080	.34972	.78688	11.187	2	.008
Pair 18	**$2Na_2O$ - $3Na_2O$**	-.1172	.02532	.01462	-.18012	-.05434	-8.021	2	.015
Pair 19	**$1SiO_2$ - $2SiO_2$**	2.2211	.28444	.16422	1.51451	2.92769	13.525	2	.005
Pair 20	**$1SiO_2$ - $3SiO_2$**	2.7299	.23756	.13715	2.13984	3.32009	19.904	2	.003
Pair 21	**$2SiO_2$ - $3SiO_2$**	.50887	.12065	.06966	.20916	.80857	7.305	2	.018
Pair 22	**1Cl - 2Cl**	-3.095	.23543	.13593	-3.68028	-2.51059	-22.773	2	.002
Pair 23	**1Cl - 3Cl**	1.2010	.31261	.18048	.42451	1.97762	6.655	2	.022
Pair 24	**2Cl - 3Cl**	4.2965	.36709	.21194	3.38461	5.20839	20.273	2	.002
Pair 25	**$1Al_2O_3$ - $2Al_2O_3$**	.28667	.11214	.06475	.00809	.56525	4.428	2	.047
Pair 26	**$1Al_2O_3$ - $3Al_2O_3$**	.45887	.10781	.06225	.19104	.72669	7.372	2	.018
Pair 27	**$2Al_2O_3$ - $3Al2O_3$**	.17220	.12654	.07306	-.14215	.48655	2.357	2	.143
Pair 28	**$1Fe_2O_3$ - $2Fe_2O_3$**	.18910	.15586	.08999	-.19808	.57628	2.101	2	.170
Pair 29	**$1Fe_2O_3$ - $3Fe_2O_3$**	.19356	.15738	.09086	-.19740	.58451	2.130	2	.167
Pair 30	**$2Fe_2O_3$ - $3Fe_2O_3$**	.00446	.00772	.00446	-.01472	.02363	1.000	2	.423
Pair 34	**1PbO - 2PbO**	-.0111	.00697	.00402	-.02840	.00620	-2.760	2	.110
Pair 35	**1PbO - 3PbO**	-.0133	.00335	.00193	-.02166	-.00501	-6.893	2	.020
Pair 36	**2PbO - 3PbO**	-.0022	.00515	.00297	-.01503	.01056	-.751	2	.531
Pair 37	**$1TiO_2$ - $2TiO_2$**	-.2400	.13123	.07577	-.56604	.08597	-3.168	2	.087
Pair 38	**$1TiO_2$ - $3TiO_2$**	.17110	.15989	.09231	-.22609	.56829	1.853	2	.205
Pair 39	**$2TiO_2$ - $3TiO_2$**	.41113	.05092	.02940	.28464	.53763	13.984	2	.005

Table 7: Statistical difference of microelements between the *Pun podi* prepared in different methods

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	SD	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	**1S - 2S**	32.6066	4.8043	2.77378	20.67204	44.54123	11.755	2	.007
Pair 2	**2S - 3S**	12.0022	2.8586	1.65041	4.90104	19.10336	7.272	2	.018
Pair 3	**2S - 3S**	-20.6044	6.5096	3.75833	-36.7752	-4.43365	-5.482	2	.032
Pair 4	**1K - 2K**	-31.5882	3.4348	1.98312	-40.1208	-23.05552	-15.92	2	.004
Pair 5	**1K - 3K**	-18.0526	1.2248	.70715	-21.0952	-15.01000	-25.52	2	.002
Pair 6	**2K - 3K**	13.5355	2.7552	1.59076	6.69107	20.38006	8.509	2	.014
Pair 7	**1P - 2P**	-2.50333	.11240	.06489	-2.78255	-2.22412	-38.57	2	.001
Pair 8	**1P - 2P**	-.98223	.22290	.12869	-1.53596	-.42851	-7.632	2	.017
Pair 9	**2P - 3P**	1.52110	.13006	.07509	1.19802	1.84418	20.258	2	.002
Pair 10	**1Mg - 2Mg**	-1.52333	.22279	.12863	-2.07676	-.96990	-11.84	2	.007
Pair 11	**1Mg - 3Mg**	.00967	.04016	.02318	-.09009	.10942	.417	2	.717
Pair 12	**2Mg - 3Mg**	1.53300	.18271	.10549	1.07913	1.98687	14.533	2	.005
Pair 13	**1Ca - 2Ca**	-.88107	.18842	.10878	-1.34913	-.41301	-8.099	2	.015
Pair 14	**1Ca - 3Ca**	-.02443	.12055	.06960	-.32389	.27502	-.351	2	.759
Pair 15	**2Ca - 3Ca**	.85663	.19296	.11141	.37730	1.33597	7.689	2	.016
Pair 16	**1Na - 2Na**	.56557	.10961	.06328	.29328	.83786	8.937	2	.012
Pair 17	**2Na - 3Na**	.40443	.06880	.03972	.23352	.57535	10.181	2	.010
Pair 18	**1Na - 3Na**	-.16113	.04859	.02805	-.28184	-.04043	-5.744	2	.029
Pair 19	**1Si - 2Si**	1.17123	.10557	.06095	.90899	1.43347	19.217	2	.003
Pair 20	**1Si - 3Si**	1.25111	.14072	.08125	.90153	1.60069	15.399	2	.004
Pair 21	**2Si - 3Si**	.07988	.04983	.02877	-.04391	.20366	2.776	2	.109
Pair 22	**1C - 2C**	-3.83100	.45316	.26163	-4.95671	-2.70529	-14.64	2	.005
Pair 23	**1C - 3C**	.96780	.11475	.06625	.68274	1.25286	14.608	2	.005
Pair 24	**2C - 3C**	4.79880	.37465	.21630	3.86812	5.72948	22.185	2	.002
Pair 25	**1Al - 2Al**	.07443	.05501	.03176	-.06222	.21108	2.344	2	.144
Pair 26	**1Al - 3Al**	.22443	.06302	.03639	.06788	.38099	6.168	2	.025
Pair 27	**2Al - 3Al**	.15000	.04409	.02546	.04048	.25952	5.893	2	.028
Pair 28	**1Fe - 2Fe**	-.00323	.01315	.00759	-.03590	.02944	-.426	2	.712
Pair 29	**1Fe - 3Fe**	.00446	.02172	.01254	-.04950	.05841	.355	2	.756
Pair 30	**2Fe - 3Fe**	.00769	.01824	.01053	-.03762	.05300	.730	2	.541
Pair 32	**1Mo - 3Mo**	-.03557	.03566	.02059	-.12416	.05303	-1.727	2	.226
Pair 33	**2Mo - 3Mo**	-.03557	.03566	.02059	-.12416	.05303	-1.727	2	.226
Pair 34	**1Pb - 2Pb**	-.02333	.00665	.00384	-.03985	-.00681	-6.077	2	.026
Pair 35	**1Pb - 3_Pb**	-.01667	.00335	.00193	-.02499	-.00834	-8.617	2	.013
Pair 36	**2Pb - 3Pb**	.00667	.00330	.00191	-.00153	.01487	3.499	2	.073
Pair 37	**1Ti - 2Ti**	.00667	.01155	.00667	-.02202	.03535	1.000	2	.423
Pair 38	**1Ti - 3Ti**	.02443	.00768	.00443	.00536	.04351	5.511	2	.031
Pair 39	**2Ti - 3Ti**	.01777	.00387	.00223	.00816	.02738	7.955	2	.015

Table 8: Statistical difference of oxide form of microelements between the *Pun podi* prepared in different seasons

One-Sample Test						
	t	Df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
1SO_3	92.025	2	.000	89.74447	85.5484	93.9405
2SO_3	85.204	2	.000	46.64443	44.2890	48.9999
3SO_3	114.096	2	.000	72.13333	69.4131	74.8535
1K_2O	43.494	2	.001	1.28777	1.1604	1.4152
2K_2O	19.024	2	.003	37.06557	28.6824	45.4487
3K_2O	22.651	2	.002	23.42447	18.9748	27.8741
1P_2O_5	6.372	2	.024	.26777	.0870	.4486
2P_2O_5	45.714	2	.000	6.25743	5.6685	6.8464
3P_2O_5	18.062	2	.003	2.79400	2.1284	3.4596
1MgO	18.673	2	.003	1.72223	1.3254	2.1191
2MgO	49.869	2	.000	4.31443	3.9422	4.6867
3MgO	14.744	2	.005	1.84557	1.3070	2.3842
1CaO	25.607	2	.002	1.28353	1.0679	1.4992
2CaO	82.329	2	.000	2.94777	2.7937	3.1018
3CaO	84.160	2	.000	1.54667	1.4676	1.6257
1Na_2O	42.266	2	.001	1.23443	1.1088	1.3601
2Na_2O	15.814	2	.004	.54890	.3996	.6982
3Na_2O	30.843	2	.001	.66613	.5732	.7591
1SiO_2	23.604	2	.002	3.31667	2.7121	3.9213
2SiO_2	46.119	2	.000	1.09557	.9934	1.1978
3SiO_2	9.645	2	.011	.58670	.3250	.8484
1Cl	7.947	2	.015	1.53443	.7037	2.3652
2Cl	22.949	2	.002	4.62987	3.7618	5.4979
3Cl	10.001	2	.010	.33337	.1899	.4768
1Al_2O_3	8.400	2	.014	.70333	.3431	1.0636
2Al_2O_3	7.264	2	.018	.41667	.1699	.6635
3Al_2O_3	6.103	2	.026	.24447	.0721	.4168
1Fe_2O_3	2.359	2	.142	.22577	-.1860	.6375
2Fe_2O_3	5.270	2	.034	.03667	.0067	.0666
3Fe_2O_3	6.637	2	.022	.03221	.0113	.0531
1PbO	1.000	2	.423	.00333	-.0110	.0177
2PbO	12.735	2	.006	.01443	.0096	.0193
3PbO	8.617	2	.013	.01667	.0083	.0250
1TiO_2	1.853	2	.205	.17110	-.2261	.5683
2TiO_2	13.984	2	.005	.41113	.2846	.5376

Table 9: Statistical difference of microelements between the *Pun podi* prepared in different periods

One-Sample Test						
	t	Df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
1S	113.588	2	.000	90.72220	87.2857	94.1587
2S	18.061	2	.003	58.11557	44.2704	71.9607
3S	92.152	2	.000	78.72000	75.0445	82.3955
1K	60.860	2	.000	1.03737	.9640	1.1107
2K	16.594	2	.004	32.62557	24.1662	41.0850
3K	27.458	2	.001	19.09000	16.0986	22.0814
1P	1.928	2	.194	.31110	-.3833	1.0055
2P	27.110	2	.001	2.81443	2.3677	3.2611
3P	38.231	2	.001	1.29333	1.1478	1.4389
1Mg	66.622	2	.000	1.02777	.9614	1.0941
2Mg	19.681	2	.003	2.55110	1.9934	3.1088
3Mg	35.569	2	.001	1.01810	.8949	1.1413
1Ca	21.339	2	.002	1.04890	.8374	1.2604
2Ca	25.689	2	.002	1.92997	1.6067	2.2532
3Ca	25.221	2	.002	1.07333	.8902	1.2564
1Na	19.388	2	.003	.91557	.7124	1.1187
2Na	9.124	2	.012	.35000	.1849	.5151
3Na	30.786	2	.001	.51113	.4397	.5826
1Si	21.154	2	.002	1.59778	1.2728	1.9228
2Si	15.209	2	.004	.42654	.3059	.5472
3Si	6.928	2	.020	.34667	.1314	.5620
1C	26.696	2	.001	1.23447	1.0355	1.4334
2C	23.259	2	.002	5.06547	4.1284	6.0025
3C	8.658	2	.013	.26667	.1341	.3992
1Al	12.962	2	.006	.28333	.1893	.3774
2Al	10.425	2	.009	.20890	.1227	.2951
3Al	4.006	2	.057	.05890	-.0044	.1222
1Fe	2.781	2	.109	.02333	-.0128	.0594
2Fe	5.304	2	.034	.02657	.0050	.0481
3Fe	3.394	2	.077	.01888	-.0051	.0428
3Mo	1.727	2	.226	.03557	-.0530	.1242
2Pb	6.077	2	.026	.02333	.0068	.0399
3Pb	8.617	2	.013	.01667	.0083	.0250
1Ti	5.511	2	.031	.02443	.0054	.0435
2Ti	7.955	2	.015	.01777	.0082	.0274

considered especially from the late inflammation until the mid-proliferation phase of wound healing[16]. Further, there are case reports of magnesium sulfate being used on patients with infected war wounds as well as ulcers resulting healing[17].

According to Lansdown 2022[18], recent discoveries revealed the ability of local calcium to modulate cell proliferation, motility/maturation, and the formation of the epidermal lipid barrier function through signal transduction and gene expression. Calcium-contained wound dressing product was widely recognized especially for the management of leg ulcers, pressure sores, and heavily exuding wounds. Further, he stated that sequential events in wound repair require a conducive environment within the wound bed and a balanced pool of metal ions, of calcium, zinc, magnesium, copper, manganese, iron, sodium, and potassium[18]. Dalisson and Barralet (2019) found in their study that decrease of calcium concentration diminishes the fibroblast adhesion and proliferation which important in wound healing[17]. Several calcium-containing dressings have been identified to facilitate wound healing. Further, Wang et al., 2014[19] stated that calcium-contained dressing material accelerated wound healing compared to Vaseline dressing by promoting the synthesis of collagen type I and wound re-epithelialization, as well as by reducing inflammation. Zhao et al., 2023[20] created another calcium-rich dressing substance and applied it to rats' wounds to speed up healing. The dressing's ability to lower inflammation and promote angiogenesis resulted in a quicker healing of the wound[21]. In situ precipitation was utilized by Jeong et al.[22] to create calcium fluoride-containing composite hydrogel dressings. They discovered that the calcium fluoride increased fibroblast and endothelial cell migration and reduced bacterial development. The calcium ion has also been demonstrated to work as a key cue, directing the cellular processes of various types of cells during wound healing in addition to being a crucial coagulation factor during hemostasis[21]. The present study showed that the *Pun podi* prepared in different methods (Table 2 and 4) and seasons (Table 1 and 3) are contained Ca in both elemental and oxide forms; therefore, it was confirmed that the *Pun podi* could be used to treat wounds more effectively.

Okaka et al., (2001)[23] found in their study that the extracellular calcium concentration played a vital role in blood coagulation and other metabolic and maintenance functions[23]. *Aspilia africana* flowers had wound healing and blood coagulation activity because of the presence of calcium in high concentrations[24]. The heavy metal analysis of *Pun podi* showed that all *Pun podi* had a considerable amount of Ca, and *Pun podi* 2 had a high amount of Ca compared to other *Pun podi* (Table 2 to 4). According to the animal study conducted with *Pun podi* 2, it showed significant wound healing activity. Therefore, the present study findings were in line with Uche Christina et al., (2017)[24]. The *Pun podi*, a traditional Siddha medicine that has been used to treat wounds since ancient times, showed Ca in both oxide and elemental form in considerable amounts.

Mahawatte et al., (2006)[25] found that K and Ca were included in all the samples prepared in different methods. The present study showed that the highest amount of K was observed in *Pun podi* 2 (32.63% ± 3.57%) while the lowest value was observed in *Pun podi* 1 (1.04% ± 0.05%). Though all *Pun podi* contained the same ingredients, the presence of microelements differed in all *Pun podi* prepared by different methods, which implied there were chemical reactions that happened during the preparation process of medicine. It should be further investigated in the future.

HPTLC is a powerful analysis tool in the field of analysis, which analyze Rf Value. It provides conclusive evidence for the identity of the compound. The Rf value can be used for the identification of the mobile phase or mobile phase ratio changes[26]. HPTLC results showed (Figure 1, 2) (Table 5), 04 spots in *Pun podi* 2, 2 spots in *Pun podi* 1, and one spot in *Pun podi* 3, at 254 nm. The values obtained from these tests were within the normal range, which indicates that the quality of the product was good because of the presence of several chemical constituents.

Conclusion

The season and method of preparation had an impact on the presence of heavy metals in *Pun podi*

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