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A Study of Biochemical Constituents of Panchakavya

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Abstract: ***Environmental and human health problems related to the use of synthetic pesticides have created an increasing pressure against their use. Organic fertilizers are sustainable, renewable bio degradable and environment friendly. Panchakavya is combination of cow's urine, milk, ghee, curd and dung. Panchakavya Therapy or Chikitsa as an alternative prophylactic or therapeutic approach for livestock and poultry health along with human health and as devotion in the services of the 'Holy Cow' which is central to the bio-chain. The analytical techniques like HPTLC,GC-MS and AAS were studied. Once biochemical constituents are known improvement could be made in natural products. They help to standardize and improve the quality of natural products. The present study reveal the spectral data of HPTLC and is very much useful for its quality improvement of panchakavya. The aim of this work was to investigate and characterize the bioactive chemical constituents by using GC-MS method. Panchakavya contain twenty peaks which indicate the presence of twenty biochemical compounds. The metals Mg, zinc, Cadmium, Cr, lead, Fe, and Cu were analyzed by using Atomic Absorption Spectrometry (AAS) in Panchakavya. The present study focused on panchakavya is to improve its quality as an organic fertilizer.***

Keywords: Panchakavya, HPTLC, GC- MS and AAS

1. Introduction

Panchakavya is a traditional method used to safeguard plants and soil micro-organisms and to increase plant production. Panchakavya is a peculiar combination of five cow products namely dung, urine, milk, curd and ghee. Organic farming in recent years is gaining impetus due to realization of inherent advantages it confers in sustaining crop production and also in maintaining dynamic soil nutrient status and safe environment (Lokanathand Parameshwarappa, 2006). The micronutrients iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) also increased in the panchakavya treated soil when compared to the other treatments. The foliar application of panchakavya had a positive influence on the four crops grown namely ragi, green gram, paddy and roslle. Soil fertility was compared to the control, and other treatments namely chemical and vermicompost (Ramya and Karpagam2017) Since the chemical fertilizers and pesticides added to the soil inhibits the growth of indigenous microorganisms of the soil. Soil microbiota is very important for the recycling of the agrowaste and in maintaining the soil fertility. Bio fertilizers have become an ideal substitute for chemical fertilizers for conditioning the soil fertility and to maintain the Agro-ecosystem (Prabu2008). In ayurvedic Vishamajvara malaria and typhoid for detoxification of body and toxic management. Use of panchakavya has been indicated for psychogenic and neurogenic disorders. Micronutrients of the soil aieds in the growth and yield of crops. Crop yield is very much influenced by soil fertility. in the present study the biochemical constituent and micronutrient content of panchakavya was studied by GC-MS and AAS (The Ayurvedic Pharmacopoeia of India 2007).

2. Materials and Method

1) **Preparation of Panchakavya :** The ingredients for panchakavya was collected from cow farm (Thiruvallur DT)) using sterile container. Based on the detailed review of literature panchakavya stock solution was prepared by using cow dung (49 Kg), cow's urine (70 L), cow's milk (21L), cow's curd (14 L) and cow's ghee (7 kg). tap water (70L). In addition, jaggery (21 Kg), tender coconut water (21 L) and ripe banana (85 Nos.) were also added as modification. The panchakavya stock solution was fermented for 30 days and is covered with a plastic mosquito net to prevent houseflies.
2) **Panchakavya extract preparation :** The sample of 10g dried powder was taken and soaked for 24h in 30 ml of methanol. The extract was filtered using Whatman filter paper No.1 , evaporated to dryness and re- dissolved in Dimethyl Sulphoxide. The extracts were preserved in airtight container and kept at 4-5 °C for further use.
3) **TLC and HPTLC:** High performance thin layer chromatography were carried out following the method Wagner and Bladt ,1996. The sample was extracted with (hexane (15µl & 25µl) and alcohol (10µl & 15µl) and applied in TLC aluminium sheet silica gel 60 F 254 (E. MERCK) and plate was developed using the solvent system Toluene: Ethyl acetate: Formic acid (8.5 : 1.5 : 0.2). After development the plate was allowed to dry in air and examined under UV – 254 nm, 366 nm and Visible light (Vanillin –Sulphuric acid).
4) **Detection of spots:** the air dried plates were viewed in ultraviolet radiation (fig-3) after treating with Vanillin Sulphuric acid . the R_f value and finger print data were recorded by CAMAG – Automatic TLC sampler, Scanner and Visualiser.
5) **Gas Chromatography –Mass Spectrometry (GC-MS) Analysis:** The GC-MS Methanol extract of panchakavya sample was performed by using PerkinElmer Clarus 500 Model and the software used is Turbomass ver 5.2. The fused silica column was packed with Elite – 5MS (5% Phenyl 95% dimethylpolysiloxane, 30 m× 250µm).The oven temperature was set up from 50°C to 280°C for 15 minutes. Helium gas (99.999%) was used as the carrier

gas at constant flow rate of 1 ml/min. An aliquot of 2 µl of sample was injected into the column with the injector temperature at 280 °C and the split ratio 10:1. The ionizing energy of 70eV was used and the electron ionization is involved. The mass range is 40-600 amu. The inlet line temperature was 200°C and source temperature was 150 °C total GC running time was 58.541 minutes. The compounds were identified referring to NIST 2005 library.

6) **Atomic Absorption of Spectrometer :** Sample of 5ml was mixed with 5ml of Conc. Nitric acid and heated slowly until the content reduced to half in a Hot plate. To the residue, 2ml of H_2O_2 were added and heated until it reduced to half of the volume. The sample was transferred to standard flask and made up to 50ml, by adding distilled water and analyzed for Mg, zinc, Cadmium, Cr, lead, Fe, and Cu. Water was taken as blank and the procedure repeated. The Atomic Absorption Spectrometry was heated with cathode lamp, the air acetylene flame was ignited and instrument was calibrated with different working standards . (Vanloon and Lichwa 1973).

3. Results and Discussion

The HPTLC finger print scanned at wavelength 254nm ,showed five polyvalent band with R_f value from 0.21 to 0.92 in which highest concentration was that of 62.97% and its R_f value was 0.21 (table 1 and fig 1). At 366nm there were five polyvalent bands with R_f value from 0.30 to 0.95 in which highest concentration was found to be a 41.71% and its R_f value was 0.30 (table 2 and fig 2).

Alcohol extract at 254nm showed six polyvalent bands with R_f value from 0.04 to 0.79 in which highest concentration was 41.09% and its R_f value was 0.04 (table 3 and fig 3). At 366nm there are six bands with R_f value of 0.04 to 0.80 in which highest concentration found to be 46.03% and its R_f value was 0.04 (table 4 and fig 4).The data will be very useful for quality control of Panchakavya and controlling batch variation (Pankaj Nariya et.al 2012). GC-MS analysis of panchakavya in methanol extract characterized showed twenty peaks which indicate the presence of twenty biochemical constituents the mass spectra identified with NIST library. The twenty compound (Table 5 and Fig 5) most prevailing compound were squalene, acetamide,2,2,2-trifluora- 64.4% , Hexadecanoic acid, methyl ester-5.95 %, Deuteroethyl n-deutero-n-ethylcarbamate-2.69%,Methyl3.beta.-hydroxy-bisnorallocholanoate-2.18%,4-Chloro-2-cyclohexyl-octahydro-benzo(e)(1,2)oxazine-3- carbonitrle2.05%,9-Octadecenoic acid,methylester,(E)-1.96%,N(.alpha.)-1.78%,4h-imidazol-4-one,3(2,6dimethylphenyl)-2-(heptafluoropropyl)-3,5-dihyro-5-methyl-1.75%,3,6Bis(phexoxyphenyl)-1,2,4,5-tetrazine-1.71%,phosphine,1,6-hexanedilylbis[diphenyl-1.51%,Ethyl(triphenylphosphoranylidene)aceytate-1.50%,[7-bromo-5-(2-chloro-phenyl)-3-(4-nitro-benzyl)-2-oxo-2,3-dihydrobenzo[e][1,4]diazepin-1-yl]-aceticacidmethyl-1.50%,4-(4-chloro-phenyl)-butyricacid-1.47%,Methylstearate-1.47%,2,2'-thiodisuccinic acid-1.44%, Cholan -24-oicacid,3-[(trifluoroacetyl)oxy]-,methylester,(3.alpha.,5.beta.)-1.40%,Acetamide,2-hydroximino-n-[3,5-bis(trifluoromethyl)phenyl]-1.39%,Undecane-1.32%,Hafnium,bis(1,3,5,7-cyclooctatetraene)-1.28%,5,6,7-trichloro-1,2,3-benzotriazin-4(3h)-one-1.24%.AAS analysis of panchakavya showed the presence of Mg-0.36ppm, zinc-2.54ppm Cadmium-4.76ppm,Cr-15.48ppm,lead-21.31ppm,Iron(Fe)-29.89ppm,andCu-55.18pp were analyzed in Panchakavya. (Table 6).

4. Conclusion

Natural products mostly contain various of chemical substances of different nature and their variations. In the present study panchakavya was analyzed for biochemical constituents by using different methods. The biochemical and micronutrients present in panchakavya attributes to the enhanced growth and yield of crops (Ramya and Karpagam 2017). Panchakavya not only enhances plant growth but also increases the fertility of the soils.

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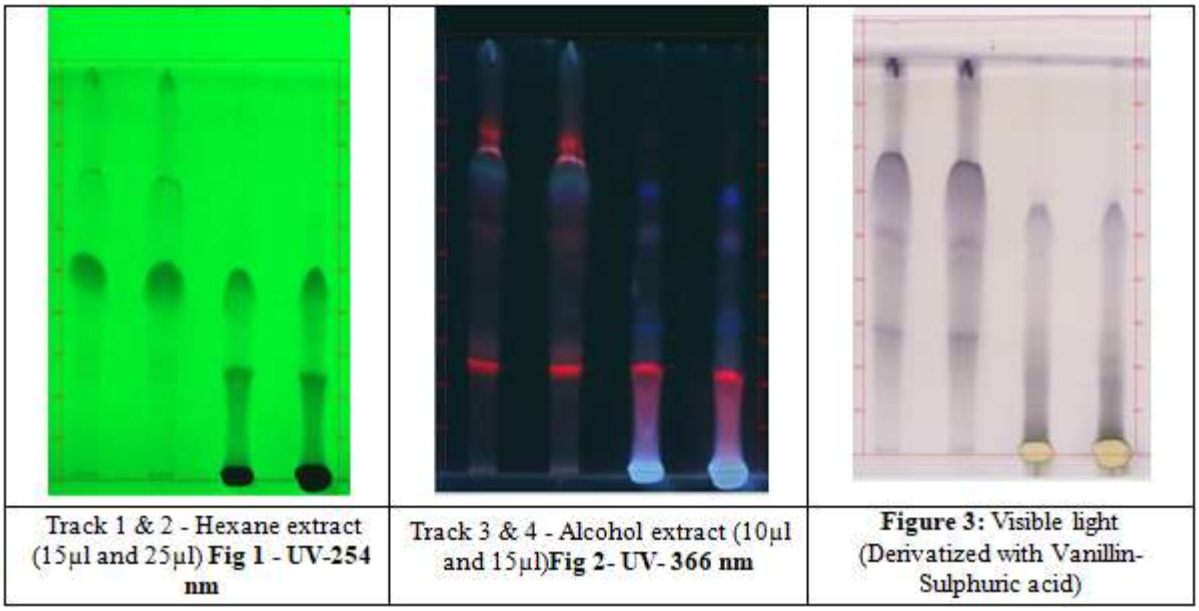

Track 1 & 2 - Hexane extract (15µl and 25µl) **Fig 1 - UV-254 nm**

Track 3 & 4 - Alcohol extract (10µl and 15µl)**Fig 2- UV- 366 nm**

Figure 3: Visible light (Derivatized with Vanillin-Sulphuric acid)

Fig 1,2 and 3 shows compound separated by TLC in Panchakavya

Table 1: Shows HPTLC finger print of Hexane extract of Panchakavya at 254nm

Peak	Start position	Start height	Max position	Max weight	Max %	End position	End height	Area	Area %
1	0.18R1	5.3AU	0.20Rf	20.7 AU	2.96%	0.21R1	12.6AU	299.1AU	0.71%
2	0.23R1	15.9 AU	0.28 Rf	55.6 AU	7.93%	0.30R1	40.0 AU	1400.8 AU	3.34%
3	0.30 R1	42.4 AU	0.32 Rf	54.9 AU	7.83%	0.36R1	37.9 AU	1927.4 AU	4.59%
4	0.39 R1	31.7 AU	0.59 Rf	441.2 AU	62.97%	0.65R1	7.9 AU	33546.8AU	79.96%
5	0.70 R1	0.7 AU	0.87 Rf	128.2 AU	18.30%	0.92R1	3.3AU	4780.5AU	11.39%

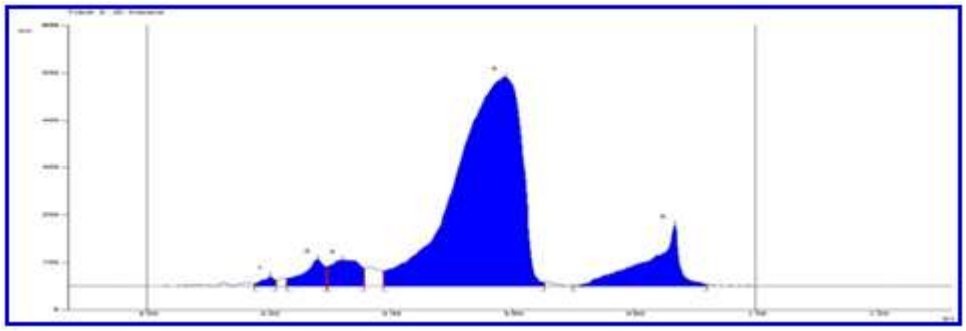

Figure 1 shows HPTLC finger print of Hexane extract of Panchakavya at 254nm

Table 2: Shows HPTLC finger print of Hexane extract of Panchakavya at 366nm

Peak	Start position	Start height	Max position	Max weight	Max %	End position	End height	Area	Area %
1	0.23Rf	13.2AU	0.28Rf	154.3 AU	41.77%	0.30Rf	36.0AU	2770.5AU	39.39%
2	0.30Rf	36.1 AU	0.32Rf	41.1AU	11.12%	0.36Rf	17.7 AU	1134.2 AU	16.12%
3	0.59 Rf	9.1 AU	0.62Rf	17.3 AU	4.68%	0.68Rf	0.1 AU	619.2 AU	8.80%
4	0.83 Rf	1.0 AU	0.87Rf	118.2 AU	32.01%	0.89Rf	33.4 AU	1574.2AU	22.38%
5	0.89 Rf	38.5 AU	0.90Rf	38.5 AU	10.41%	0.95Rf	8.8AU	935.7AU	13.30%

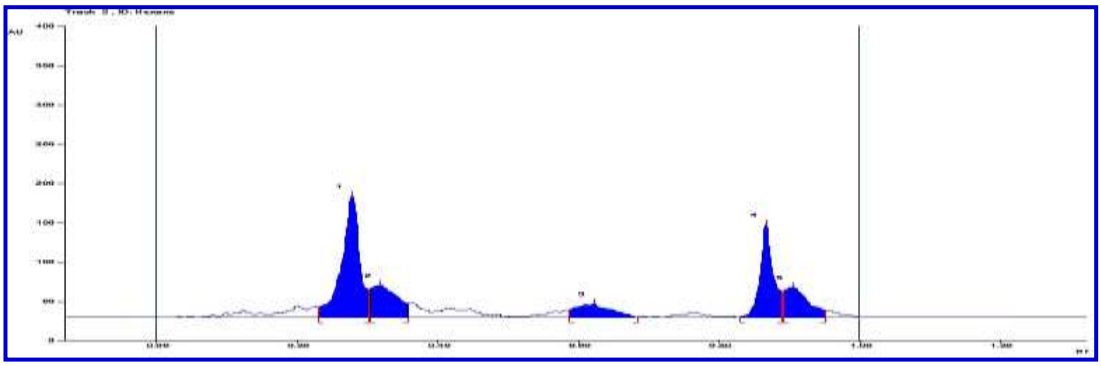

Figure 2 Shows HPTLC finger print of Hexane extract of Panchakavya at 366nm

Table 3: Shows HPTLC finger print of Alcohol extract of Panchakavya at 254nm

Peak	Start position	Start height	Max position	Max weight	Max %	End position	End height	Area	Area %
1	0.00R1	24.5AU	0.03R1	171.7AU	18.71%	0.04Rf	1.0AU	2024AU	4.72%
2	0.14R1	12.7 AU	0.29 R1	234.0AU	26.87%	0.32Rf	0.4 AU	12025.9 AU	29.06%
3	0.35 R1	8.0 AU	0.40 R1	79.6AU	8.16%	0.42Rf	75.1 AU	1746.0AU	4.06%
4	0.42 R1	74.8 AU	0.57 R1	357.4 AU	41.09%	0.69Rf	0.1AU	28487.3AU	81.81%
5	0.69 R1	0.3 AU	0.74 R1	13.9 AU	1.60%	0.75Rf	10.8AU	363.4AU	0.35%
6	0.75R1	11.1AU	0.78R1	14.2AU	1.69%	0.79Rf	0.2AU	206.3AU	0.48%

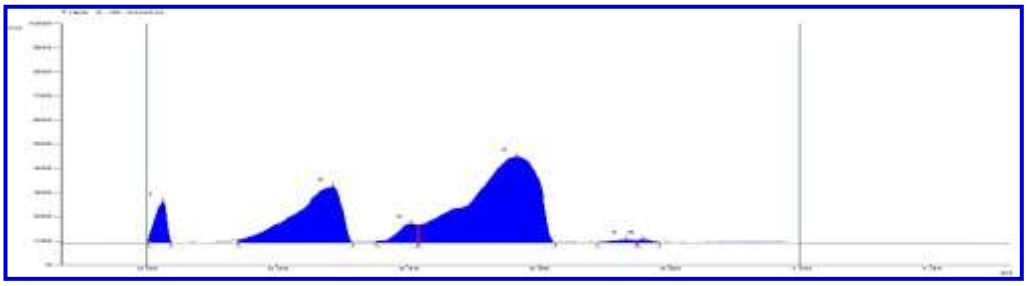

Figure 3: Shows HPTLC finger print of Alcohol extract of Panchakavya at 254nm

Table 4: Shows HPTLC finger print of Alcohol extract of Panchakavya at 366nm

Peak	Start position	Start height	Max position	Max weight	Max %	End position	End height	Area	Area %
1	0.00Rf	18.0AU	0.02Rf	265.0AU	48.03%	0.04Rf	0.9AU	3034.1AU	29.28%
2	0.14Rf	6.9 AU	0.22 Rf	55.2AU	10.01%	0.23Rf	50.4 AU	1627.3 AU	17.64%
3	0.24Rf	51.2 AU	0.26 Rf	151.9AU	27.54%	0.29Rf	2.2 AU	2910.4AU	28.09%
4	0.34 Rf	20 AU	0.40 Rf	19.0 AU	3.45%	0.43Rf	8.7AU	678.3AU	6.55%
5	0.59 Rf	9.4 AU	0.63 Rf	40.4 AU	7.33%	0.69Rf	0.1AU	1409.8AU	13.61%
6	0.72Rf	1.9AU	0.76Rf	20.1AU	3.64%	0.60Rf	1.6AU	501.0AU	4.64%

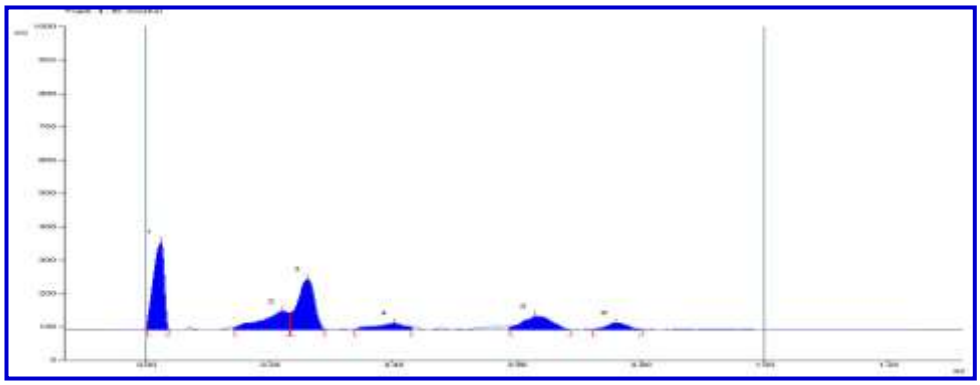

Figure 4: Shows HPTLC finger print of Alcohol extract of panchakavya at 366nm

Table 5: Shows Biochemical constituents of GC-MS in Panchakavya

Peak#	R.Time	Area%	Name	Molecular weight g/mol	Molecular formula
1	4.022	1.32	Undecane	156.31	$C_{11}H_{24}$
2	141.191	1.50	Ethyl (triphenylphosphoranylidene)aceytate	348.37	$C_{22}H_{21}O_2$ P
3	14.550	1.75	4h-imidazol-4-one.,3-(2,6-dimethylphenyl)-2-(heptafluoropropyl)-3,5-dihyro-5-methyl	-	-
4	21.421	64.41	Acetamide,2,2,2-trifluora-	219.163	C_9H_8 F_3NO_2
5	23.100	1.28	Hafnium,bis(1,3,5,7-cyclooctatetraene)-	104.15	C_8H_8
6	25.452	1.39	Acetamide,2-hydroximino-n-[3,5-bis(trifluoromethyl)phenyl]-	325.167Da	$C_{11}H_5$ $F_6N_3O_2$
7	25.881	1.24	5,6,7-trichloro -1,2,3-benzotriazin-4(3h)-one	-	-
8	33.194	5.95	Hexadecanoic acid, methyl ester	270.457	$C_{17}H_{34}O_2$
9	37.667	1.47	methyl stearate	298.511	$C_{19}H_{38}O_2$
10	38.004	1.96	9-Octadecenoic acid,methyl ester,(e)-	296.4879	C_3H_8O
11	39.771	1.50	[7-bromo-5-(2-chloro-phenyl)-3-(4-nitro-benzyl)-2-oxo-2,3-dihydro-benzo[e][1,4]diazepin -1-yl]-acetic acid methyl	-	-
12	53.335	1.44	2,2'-Thiodisuccinic acid	-	-
13	53.458	1.78	N(.alpha.)-benzoyloxycarbonyl-n(.beta.)-trimethylammonio-1-alanine,inner sal	89.094	$C_3H_7NO_2$
14	53.510	1.51	Phosphine,1,6-hexanedilylbis[diphenyl-	454.522602	$C_{30}H_{32}P_2$
15	53.546	1.71	3,6-bis(p-n-hexoxyphenyl)-1,2,4,5-tetrazine	-	-
16	53.631	2.69	Deuteroethyl n-deutero-n-ethylcarbamate	-	-
17	56.390	2.18	Methyl 3.beta.-hydroxy-bisnorallocholanoate	118.132	$C_5H_{10}O_3$

18	56.493	2.05	4-chloro-2-cyclohexyl-octahydro-benzo(e)(1,2)oxazine-3-carbonitrle	-	-
19	56.557	1.40	Cholan -24-oic acid,3-[(trifluoroacetyl)oxy]-,methyl ester, (3.alpha.,5.beta.)-	-	-
20	58.541	1.47	4-(4-Chloro-phenyl)-butyric acid	198.646Da	$C_{10}H_{11}C10_2$
		100.0			

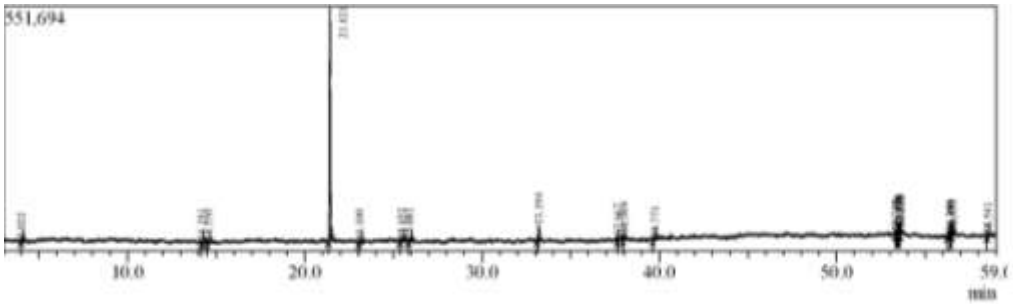

Figure 5: Shows Biochemical constituents of GC-MS in Panchakavya

Table 6: Shows Atomic Absorption Spectrophotometer Metal content of panchakavya

S.No	Metals	Concentration (ppm)
1	Magnesium	0.36
2	Zinc	2.54
3	Cadmium	4.76
4	Chromium	15.48
5	Lead	21.31
6	Iron	29.89
7	Zinc	2.54

Author Profile

Ramya Viswanathan Ph.d Research scholar under the guidance of **Dr. S. Karpagam** from Queen Mary's College, Chennai-04, Tamil Nadu. This is my 5th Paper Publications. I very much interested in working on Panchakavya. Panchakavya it's an organic fertilizer and very useful economically profitable for all crops. I Dedicated to my **Parents: M. Viswanathan, V. Sarasuwathy** my **Beloved Guide Dr. S. Karpagam** and **God Almighty**.