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Research Article

A COMPARATIVE PHYTOCHEMICAL ANALYSIS AND HPTLC FINGERPRINTING OF ARDRAKA AND SHUNTI IN DIFFERENT DOSAGE FORMS AND ITS RELATION TO DIABETES

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ABSTRACT

Ginger is a household spice used globally for different purposes. As per Ayurveda, it is considered to possess therapeutic properties for various ailments. Although ginger has *Pramehagna* (anti-diabetic) property, there are very few formulations for diabetes that contain this as an ingredient. However, some pharmaceutical and clinical studies have shown significant anti-diabetic property of ginger. According to Ayurveda, *Ardraka* (fresh rhizome) and *Shunti* (dry rhizome) have different properties and are widely used in many formulations. To analyze the difference between both forms of ginger in varied forms, phytochemical screening, and HPTLC study was done on *Ardraka swarasa* (juice of fresh rhizome), *Shunti churna* (powder), *Ardraka* and *Shunti Kashaya* (decoction of fresh and dry ginger), *Ardraka* and *Shunti Hima* (cold infusion of fresh and dry ginger), *Ardraka* and *Shunti phanta* (cold decoction of fresh and dry ginger). Alkaloids were present abundantly in *Ardraka swarasa, Ardraka kashaya, Shunti kashaya, Shunti hima* and *Shunti phanta*. Flavonoids were present in excess only in *Ardraka swarasa*. HPTLC analysis showed more peaks in *Kashaya* of both forms of rhizomes and *Ardraka phanta*.

INTRODUCTION

Ginger is an integral part of Indian cuisine which is commonly used in many dishes. Japan uses pickled ginger slices called *Gari* as a condiment, sliced ginger with sugar to make tea and ginger is commonly used in Western countries to flavor cookies and cakes [1]. It is also used as a home remedy for various disorders. Ginger is called *Ardraka* (wet ginger) and *Shunti* (dry ginger) in Sanskrit and has been extensively used in Ayurveda medicine. It is also referred to as "*Mahabheshaja*"[2] (abundance of medicinal property) and "*Vishwabheshaja*"[2] (universal medicine that can be used in all age groups for all diseases) signifying its magnitude of therapeutic potency. It is used as *Ekamoolika prayoga* (single drug prescription to treat and prevent disease) in various disorders like *Agnimandya* (loss of appetite), *Aruchi* (loss of taste), *Kasa* (cough), *Shwasa* (dyspnoea), *Hikka* (hiccup), *Amavata* (rheumatoid arthritis), *Kati shoola* (Back pain), *Shotha* (oedema), *Hridroga* (heart disorders), *Sheetapitta* (allergic rhinitis), *Karna shoola* (ear ache)[3]. Ginger is the main ingredient in various compound formulations like *Ardraka khanda* and *Soubhagya shuntipaka*.

Although both *Ardraka* and *Shunti* exhibit some similar properties, they also vary in particular *Gunas* (property). Both have *Katu rasa* (pungent taste), *Ushna veerya* (hot potency), *Madhura vipaka* (sweet in post digestive state) and *Kaphavatashamaka* (subsides *Kapha* and *Vata dosha*) properties. But *Ardraka* is *Guru* (heavy), *Rookshsa* (dry) and *Teekshna* (sharp) and it does *Bhedana* (strong laxative) and is used in *Aanaha* (flatulence), *Shoola* (pain) and *Vibandha*[4] (constipation) whereas *Shunti* is *Snigdha* (unctuous), *Mala graahi* (absorption) and *Vayu vibandhanut* (obstructs *Vata dosha*)[5]. *Ardraka* is found in *Ganas* (group of drugs with similar properties) like *Pippalyadi*[6], *Deepaniya* and *Shoolaprashamana ganas*[7] whereas *Shunti* is found in *Triptighna*, *Arshoghna*, *Trishnanigrahana ganas*[8].

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The fundamentals of Ayurveda pharmaceuticals are *Panchavidha kashaya kalpana* (five dosage forms). These preparations were designed to get the best extractions of the drug's potency depending on the nature of the herb. *Ardraka* and/or *Shunti* can be converted into *Swarasa* (juice), *Kwatha* (decoction), *Hima* (cold infusion), *Phanta* (hot infusion) and *Churna* (powder).

Diabetes is soaring up to an alarming epidemic level [9]. Despite the advancements in the management of diabetes, an apt treatment algorithm has not yet been achieved to manage the disorder or reduce the progression of complications arising from this disorder. Ayurveda has various anti-diabetic treatment modalities and mentions several formulations which are useful to treat diabetes. One among them is *Gudardraka prayoga* which contains *Shunti* as an ingredient[10]. Although not many anti-diabetic formulations contain ginger, extensive research on its phytoconstituents and clinical studies have proved to significantly reduce blood glucose levels. Hence, to evaluate the presence and magnitude of extraction of different phytoconstituents quantitatively from wet and dry ginger in *Pancha vidha kashaya kalpana*, this study was taken up.

MATERIALS AND METHODS

Collection of Raw Materials

Ardraka and *Shunti* were collected from the local market in Bengaluru and authenticated by the Botanist at Survey of Medicinal Plant Unit, Central Ayurveda Research Institute, Bengaluru. *Ardraka* was washed properly in tap water to remove the foreign materials.

Preparation of different dosage forms

The fundamentals of Ayurvedic dosage form namely *Swarasa* (for *Ardraka*), *Kashaya*, *Hima*, *Phanta* and *Churna* (for *Shunti*) were prepared as per *Sharangadhara samhita*[11] reference.

Preparation of *Swarasa*: 10g of cleaned *Ardraka* was taken in a mortar pestle and pounded well and squeezed in hand to extract its juice. This procedure was repeated until the ginger became tasteless.

Preparation of *Kashaya*: 10g each of *Ardraka* and *Shunti* was crushed and boiled separately with 40ml of water in medium flame to reduce it to 10ml. It was then filtered to obtain the *Kashaya*.

Preparation of *Hima*: 10g each of *Ardraka* and *Shunti* was taken separately, crushed and 60ml of normal water was added to both. This was kept overnight and filtered in the morning after maceration.

Preparation of *Phanta*: 10g each of *Ardraka* and *Shunti* was pounded separately to which 40 ml of boiling water was added. It was stirred well and filtered to obtain *Phanta*.

Preparation of *Churna*: 10g of *Shunti* was pounded and sieved through a muslin cloth. The obtained powder was stored until use.

Phytochemical Analysis

The presence of alkaloids, flavonoids, tannins, glycosides, proteins, and carbohydrates was confirmed by phytochemical tests for different dosage forms of *Ardraka* and *Shunti*.

i. Test for Alkaloids

Dragendorff's test: 2ml of different extracts were taken in a test tube and Dragendorff's reagent was added to it. An orange-red precipitate indicates the presence of alkaloids.

ii. Test for Flavonoids

Alkaline reagent test: To 2ml of the extract, 3ml of sodium hydroxide was added. A deep yellow colour appeared. The solution became colourless by adding a few drops of dilute HCl indicating the presence of flavonoids.

iii. Test for glycosides

2ml of the extract was dissolved in 1ml of glacial acetic acid containing one drop of ferric chloride solution. Concentrated sulphuric acid was added along the sides of the test tube. The appearance of a brown ring at the junction of two liquids indicated the presence of glycosides.

iv. Test for Proteins

Ninhydrin test

To 1ml of extract, 2 drops of freshly prepared 0.2% ninhydrin solution was added. Purple colour indicates the presence of proteins.

v. Test for Carbohydrates

Molish test

To 2ml of the extract, a few drops of alcoholic α-naphthol solution was added. Later, a few drops of concentrated H_2SO_4 were added along the sides of the test tube. Violet-coloured ring appeared at the junction of two liquids indicating the presence of carbohydrates.

Sample preparation for HPTLC

All the samples were taken in a petri dish and evaporated in the water bath until the residue was formed. This residue was used for HPTLC analysis. 500mg of each sample was weighed and added with 5ml of water. The samples were sonicated for 15 minutes, centrifuged and filtered.

HPTLC Profile

HPTLC analysis was carried out using the CAMAG HPTLC system with an automatic sample applicator. TLC Silica gel 60 F254 (20 x 10cm) was used for sample application. Based on the literature study, the solvent system was chosen to be Toluene: ethyl acetate (3:1) [12]. After application, the plate was transferred to the twin-trough chamber of width

20x10cm, where the solvent system was pre-saturated for 20 minutes. The photo-documentation was done in white light, 254nm and 366nm with the help of TLC Visualizer 2. The number of peaks, the area under the curve and Rf values were noted. The whole investigation process was carried out in an air-conditioned room maintained at 25°C.

RESULTS AND DISCUSSIONS

In the Zingiberaceae family, especially *Zingiber officinale*, it is generally believed that secondary metabolites produced by the plants are transported to the rhizomes where they accumulate [13]. This implies rhizomes are rich in secondary metabolites than other parts of the plant which may or may not be retained in them when used in other forms for consumption. To understand the difference between these phyto-constituents in various dosage forms, an analysis was conducted. Ayurveda texts emphasize only *Swarasa*, *Churna* and *Kashaya* of ginger but *Hima* and *Phanta* preparation of ginger is a common household preparation carried out as a home remedy for various ailments. We can also find many studies on ginger-infused water which is similar to *Hima kalpana*. In a study, it was found that ginger soaked for 12 hours contained the highest anti-oxidant property than ginger soaked for 6 and 9 hours[14]. Similarly, a study was done to determine the anti-oxidant property and phenolic content of ginger in decoction form and infused extraction method where hot water is added to ginger powder which is comparable to *Phanta kalpana* [15].

In this study, the phyto chemical screening of *Ardraka* revealed the presence of alkaloids in abundance in *Ardraka swarasa*, *Ardraka kashaya*, *Shunti kashaya*, *Shunti hima* and *Shunti phanta*. Few alkaloids are not heat sensitive. They can be extracted at high temperatures [16]. Hence it was seen in both forms of *Kashaya*. Alkaloids alter the activities of different enzymes related directly or indirectly to carbohydrate metabolism to control glucose levels in the body. [17] They promote glucose consumption and glycogen synthesis [18]. Flavonoids were present in abundance only in *Ardraka swarasa*. Flavonoids are heat sensitive [19]. Direct heating can destroy enzyme activity and block the synthesis pathway of flavonoids[20]. Hence, Kashaya and Phanta had only traces of flavonoids. They also have low solubility in water and, therefore were not extracted in *Hima*[21]. However, it was also detected in traces in *Shunti churna*. Flavanoids display anti-diabetic action by supporting the regulation of carbohydrate metabolism, insulin signaling, insulin secretion, glucose uptake, and adipose deposition [22]. They target multiple molecules that are involved in the regulation of several pathways, like improving β-cell proliferation, promoting insulin secretion, reducing apoptosis, and improving hyperglycemia by regulating glucose metabolism in the liver. [23] Table 1 and Table 2 show the phyto chemical screening of both rhizomes.

Table 1: Phyto-Chemical Constituents of *Ardraka*

	A1	A2	A3	A4
Alkaloids	++	++	-	-
Flavonoids	++	+	+	+
Glycosides	+	+	+	+
Proteins	-	-	-	-
Carbohydrates	+	+	+	+

A1: Swarasa, A2: Kashaya, A3: Hima, A4: Phanta

- : absent, +: traces, ++ : abundance

Table 2: Phyto-Chemical Constituents of *Shunti*

	S1	S2	S3	S4
Alkaloids	+	++	++	++
Flavonoids	+	+	+	+
Glycosides	+	+	+	=
Proteins	-	-	-	-
Carbohydrates	+	+	+	+

S1: Swarasa, S2: Kashaya, S3: Hima, S4: Phanta

- : absent, +: present, ++ : abundance

HPTLC

The HPTLC analysis was carried out to understand the difference in extraction between different dosage forms of *Ardraka* and *Shunti* in terms of Rf value, number of peaks, area of the peaks and intensity of the bands. Fig 1 represents the TLC plate at 254nm & 366nm with all dosage forms of *Ardraka* and *Shunti.* The number of peaks and peak area are not the same. So, through HPTLC analysis, the fingerprinting of different dosage forms of *Ardraka* and *Shunti* has been achieved. In the densitometric scan, at 254nm, the *Ardraka* showed 3, 4, 2 and 4 peaks for S*warasa, Kashaya, Hima* and *Phanta* respectively (Fig 2). *Shunti* showed 2, 4, 2 and 3 peaks for *Churna, Kashaya, Hima* and *Phanta* respectively (Fig 3).

At 366 nm, *Ardraka* showed 1, 4, 1 and 2 peaks for *Swarasa*, *Kashaya*, *Hima* and *Phanta* respectively (Fig 4). *Shunti* showed 2, 2, 0 and 1 peaks for *Churna*, *Kashaya*, *Hima* and *Phanta* respectively (Fig 5). Fig 6 & Fig 7 shows the comparative number of peaks of all dosage forms of *Ardraka* and *Shunti* at 254nm and 366nm in a concise form. Table 3 and Table 4 exhibit the Rf values of the peaks obtained and Fig 8 and Fig 9 depict about number of peaks.

Table 3: Rf Values of Different Dosage Forms at 254 Nm

Ginger form	Rf of *Swarasa/ Churna*	Rf of *Kashaya*	Rf of *Hima*	Rf of *Phanta*
Ardraka	0.308 0.608 0.865	0.097 0.313 0.611 0.861	0.303 0.608	0.016 0.087 0.244 0.305
Shunti	0.621 0.874	0.0198 0.148 0.618 0.879	0.098 0.147	0.148 0.610 0.869

Table 4: Rf Values of Different Dosage Forms at 366 nm

366 nm	*Swarasa/churna*	*Kashaya*	*Hima*	*Phanta*
Ardraka	0.60	0.034 0.347 0.598 0.631	0.597	0.242, 0.627
Shunti	0.444 0.610	0.532 0.603	No peaks	0.603

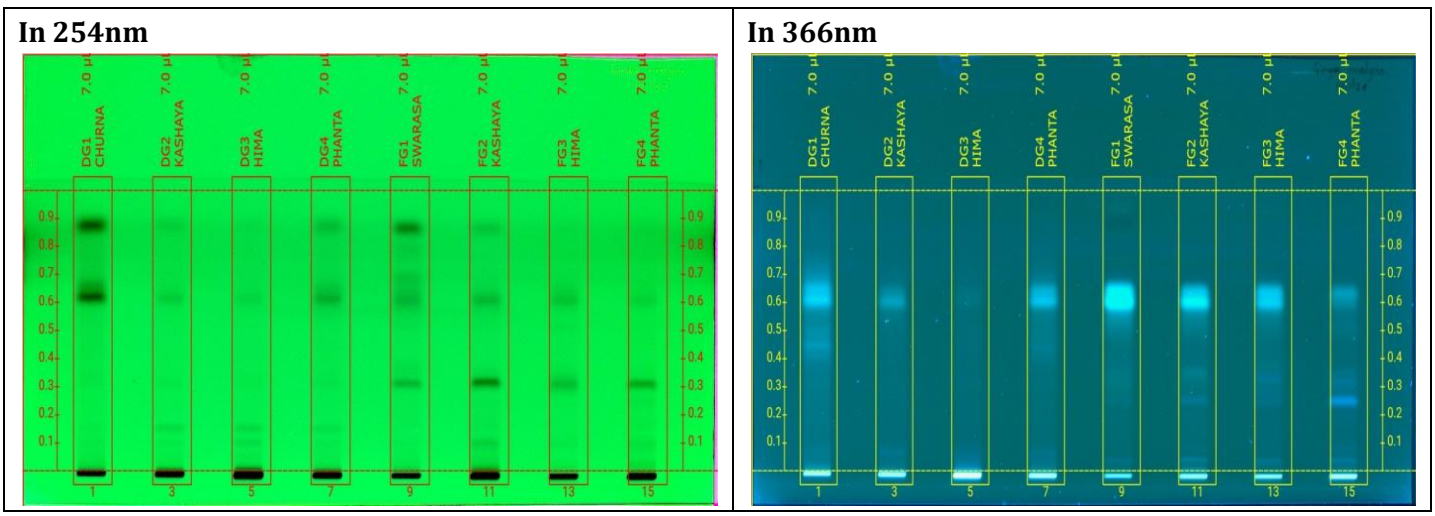
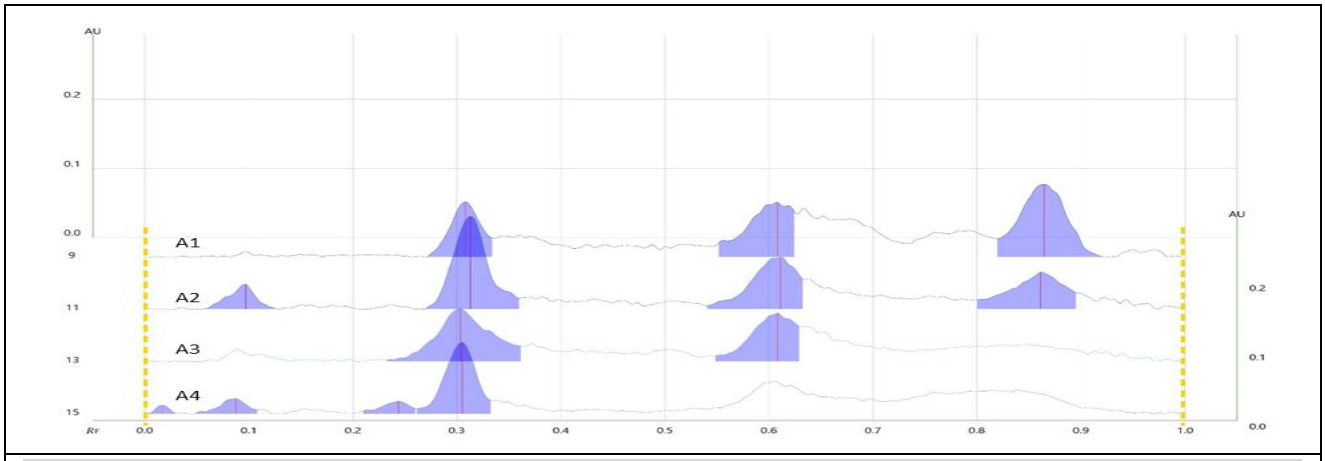


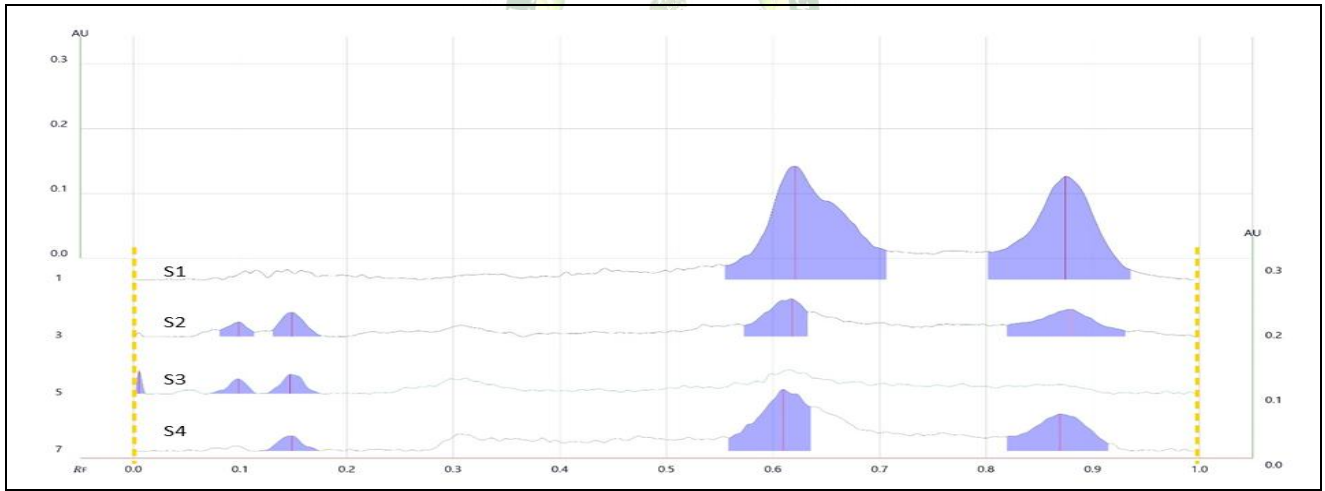
Fig 1: TLC of *Ardraka* and *Shunti* of all dosage forms at 254nm and 366nm
DG –*Shunti*, FG –*Ardraka*



Track	Max RF	Max pos. (mm)	Height	Height %	Start RF	Start pos. (mm)	Start height	End RF	End pos. (mm)	End height	Area	Area %
9	0.308	27.100	0.080	30.237	0.269	24.700	0.000	0.335	28.800	0.026911	0.002770	22.455
9	0.608	45.700	0.079	29.939	0.552	42.200	0.020	0.626	46.800	0.063119	0.003991	32.350
9	0.865	61.600	0.105	39.825	0.819	58.800	0.026	0.923	65.200	0.000047	0.005575	45.195
11	0.097	14.000	0.037	12.193	0.053	11.300	0.000	0.127	15.900	0.000036	0.001083	8.004
11	0.313	27.400	0.134	44.803	0.260	24.100	0.000	0.361	30.400	0.016028	0.005384	39.789
11	0.611	45.900	0.075	25.085	0.540	41.500	0.005	0.634	47.300	0.042785	0.003882	28.690
11	0.861	61.400	0.054	17.919	0.800	57.600	0.014	0.897	63.600	0.023539	0.003182	23.516
13	0.303	26.800	0.077	52.268	0.232	22.400	0.003	0.363	30.500	0.021569	0.004713	57.609
13	0.608	45.700	0.070	47.732	0.548	42.000	0.008	0.631	47.100	0.050780	0.003468	42.391
15	0.016	9.000	0.013	8.024	0.002	8.100	0.000	0.031	9.900	0.000935	0.000190	3.643
15	0.087	13.400	0.022	14.062	0.048	11.000	0.001	0.110	14.800	0.006278	0.000702	13.449
15	0.244	23.100	0.018	11.766	0.210	21.000	0.004	0.261	24.200	0.007761	0.000595	11.398
15	0.305	26.900	0.104	66.148	0.261	24.200	0.008	0.334	28.700	0.019035	0.003731	71.510

Fig 2: Densitometric scan of *Ardraka* at 254nm

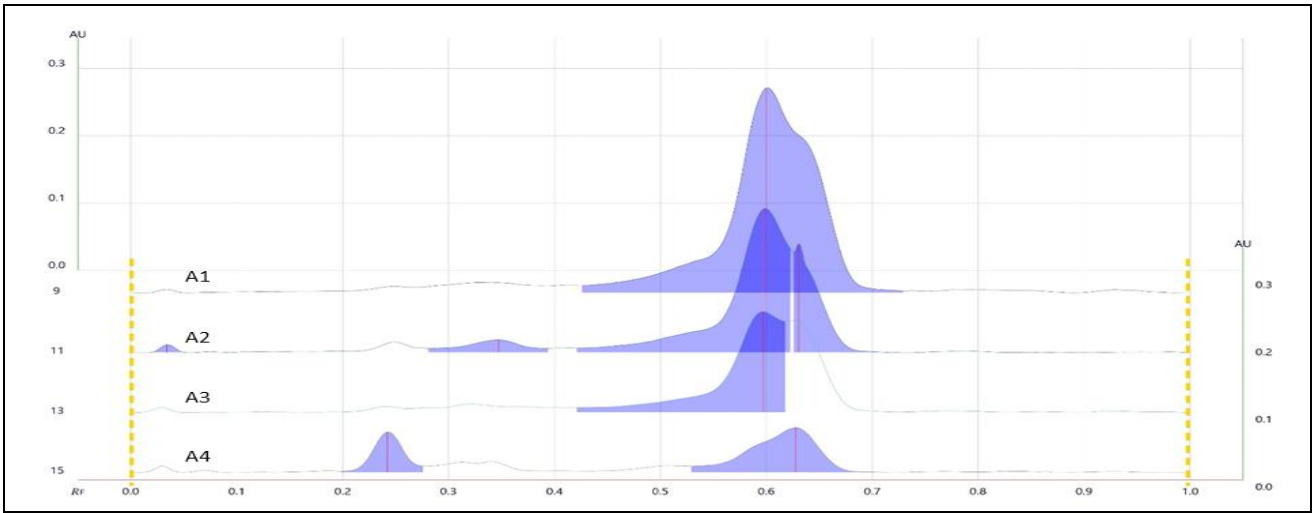
A1: Ardraka swarasa, A2: Ardraka kashaya, A3: Ardraka Hima, A4: Ardraka phanta



Track	Max RF	Max pos. (mm)	Height	Height %	Start RF	Start pos. (mm)	Start height	End RF	End pos. (mm)	End height	Area	Area %
1	0.621	46.500	0.175	52.328	0.555	42.400	0.022	0.707	51.819	0.044991	0.014221	55.371
1	0.874	62.200	0.160	47.672	0.802	57.696	0.044	0.937	66.075	0.014238	0.011462	44.629
3	0.098	14.100	0.023	14.190	0.081	13.000	0.010	0.115	15.100	0.007137	0.000532	7.546
3	0.148	17.200	0.038	23.290	0.130	16.052	0.011	0.177	18.990	0.001773	0.001017	14.411
3	0.618	46.300	0.059	36.221	0.573	43.500	0.017	0.633	47.254	0.037556	0.002557	36.243
3	0.879	62.500	0.043	26.299	0.819	58.800	0.018	0.932	65.800	0.007907	0.002949	41.800
5	0.005	8.300	0.036	40.519	0.002	8.100	0.000	0.013	8.800	0.000000	0.000189	12.967
5	0.098	14.100	0.023	25.829	0.068	12.200	0.000	0.118	15.300	0.000438	0.000491	33.722
5	0.147	17.100	0.030	33.651	0.118	15.300	0.000	0.177	19.000	0.000000	0.000777	53.311
7	0.148	17.200	0.024	13.377	0.115	15.100	0.000	0.176	18.900	0.000000	0.000624	6.741
7	0.610	45.800	0.095	54.169	0.558	42.600	0.020	0.637	47.500	0.068085	0.005001	54.018
7	0.869	61.900	0.057	32.454	0.819	58.800	0.022	0.916	64.800	0.010657	0.003633	39.240

Fig 3: Densitometric scan of *Shunti* at 254 nm

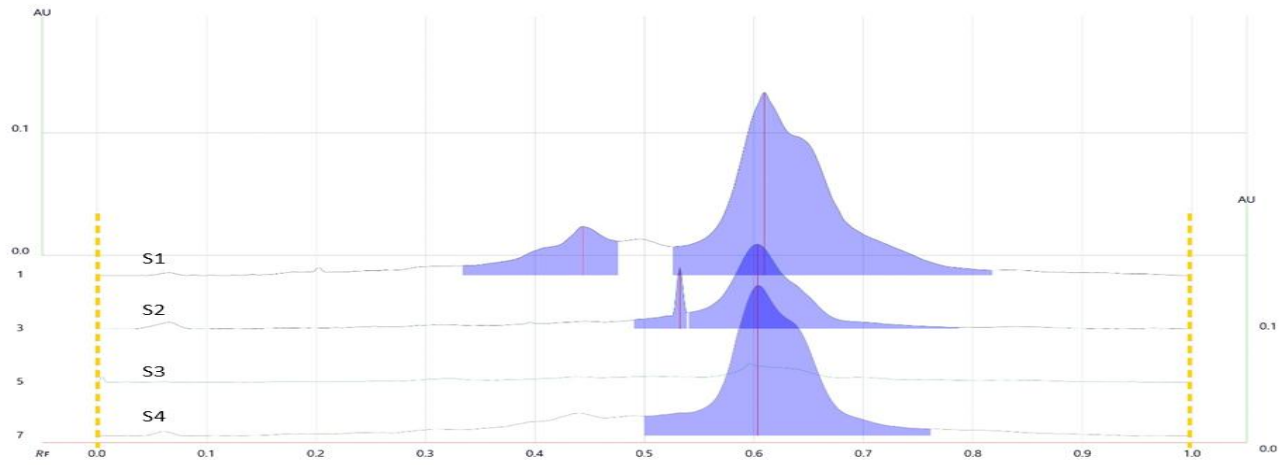
S1: Shunti churna, S2: Shunti kashaya, S3: Shunti hima, S4: Shunti phanta



Track	Max RF	Max pos. (mm)	Height	Height %	Start RF	Start pos. (mm)	Start height	End RF	End pos. (mm)	End height	Area	Area %
9	0.600	45.200	0.305	100.000	0.426	34.400	0.011	0.731	53.300	0.00268	0.02760	100.000
11	0.034	10.100	0.012	2.900	0.018	9.100	0.000	0.053	11.300	0.00000	0.00019	1.019
11	0.347	29.500	0.019	4.706	0.281	25.400	0.006	0.395	32.500	0.00629	0.00128	6.862
11	0.598	45.100	0.214	52.616	0.421	34.100	0.007	0.624	46.700	0.15063	0.01289	69.084
11	0.631	47.100	0.162	39.778	0.626	46.800	0.148	0.718	52.500	0.00016	0.00430	23.035
13	0.597	45.000	0.151	100.000	0.421	34.100	0.007	0.619	46.400	0.13540	0.00939	100.000
15	0.242	23.000	0.060	47.467	0.198	20.300	0.002	0.277	25.200	0.00928	0.00205	29.903
15	0.627	46.900	0.066	52.533	0.529	40.800	0.009	0.711	52.100	0.00001	0.00480	70.097

Fig 4: Densitometric scan of *Ardraka* at 366nm

A1: Ardraka swarasa, A2: Ardraka kashaya, A3: Ardraka Hima, A4: Ardraka phanta



Track	Max RF	Max pos. (mm)	Height	Height %	Start RF	Start pos. (mm)	Start height	End RF	End pos. (mm)	End height	Area	Area %
1	0.444	35.500	0.040	21.092	0.334	28.700	0.008	0.477	37.600	0.02798	0.00311	17.094
1	0.610	45.800	0.149	78.908	0.526	40.600	0.023	0.819	58.800	0.00416	0.01506	82.906
3	0.532	41.000	0.050	42.059	0.490	38.400	0.007	0.540	41.500	0.01297	0.00074	12.534
3	0.603	45.400	0.069	57.941	0.540	41.500	0.013	0.789	56.900	0.00125	0.00519	87.466
7	0.603	45.400	0.123	100.000	0.500	39.000	0.016	0.763	55.300	0.00555	0.01102	100.000

Fig 5: Densitometric scan of *Shunti* at 366nm

S1: Shunti Churna, S2: Shunti Kashaya, S3: Shunti Hima, S4: Shunti Phanta

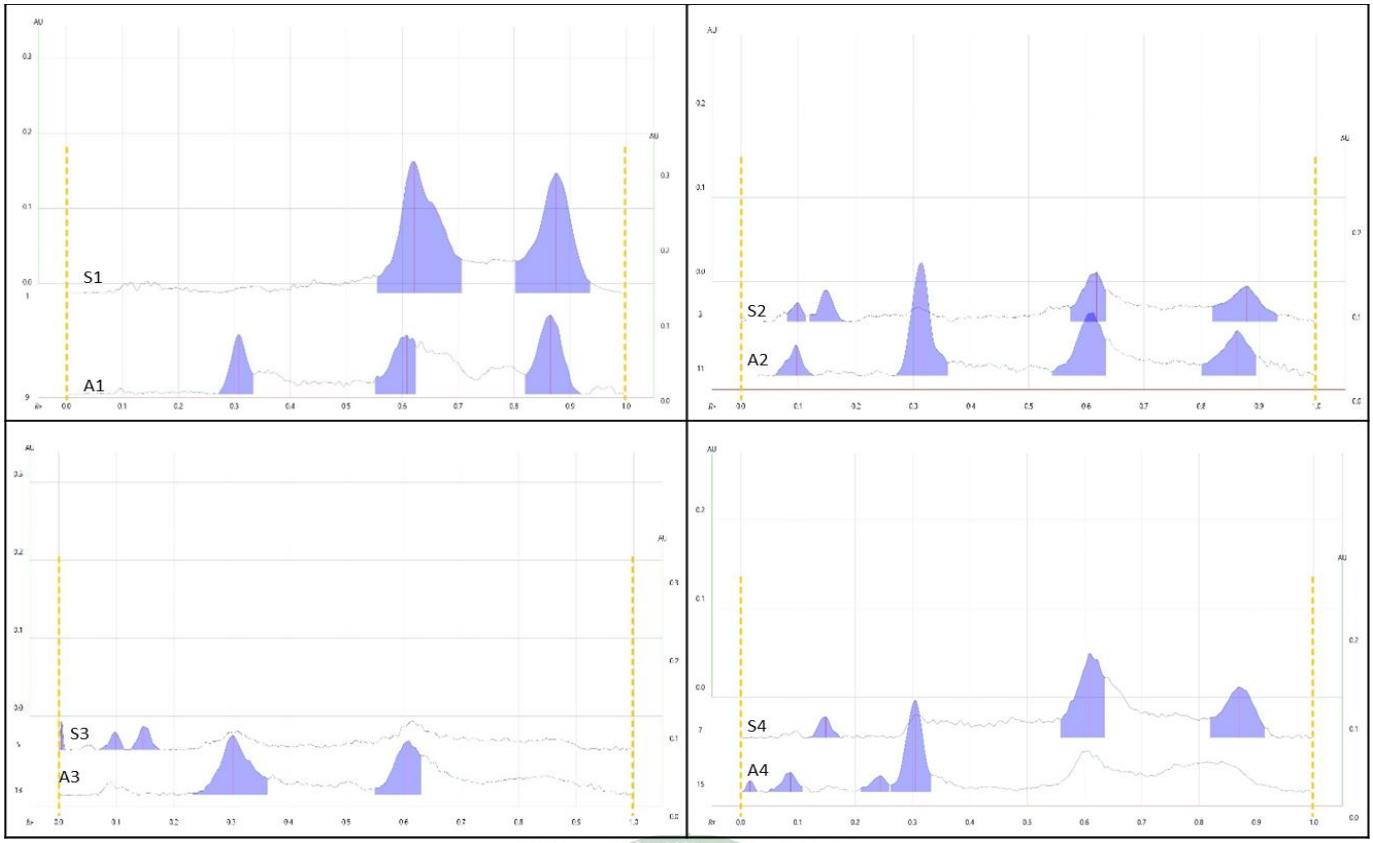


Fig 6: Comparison of peaks of both forms of ginger at 254 nm

A1: Swarasa, S1: Swarasa, A2: Kashaya, S2: Kashaya, A3: Hima, S3: Hima, A4: Phanta, S4: Phanta

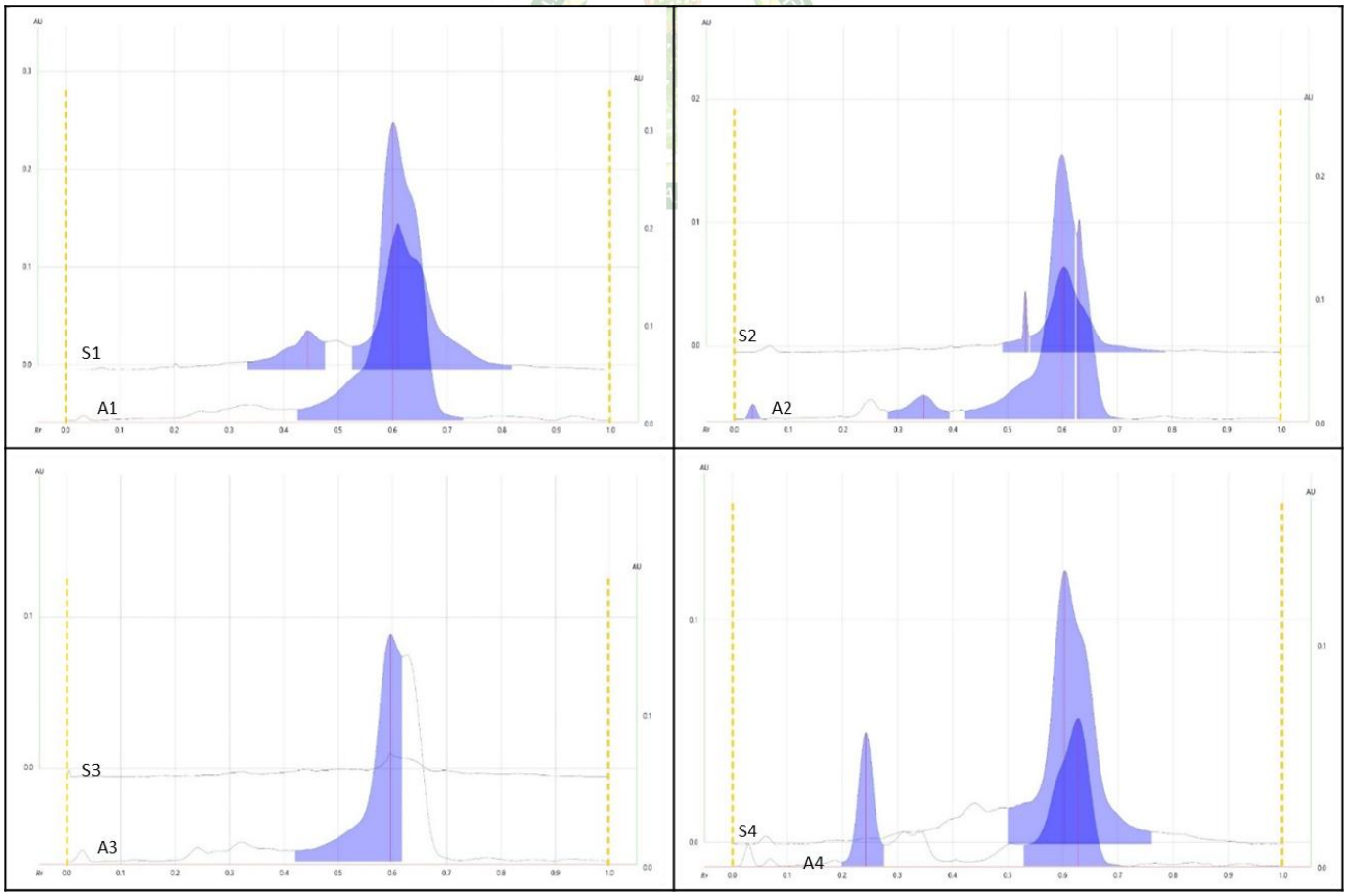


Fig 7: Comparison of peaks of both forms of ginger at 366 nm

A1: Swarasa, S1: Swarasa, A2: Kashaya, S2: Kashaya, A3: Hima, S3: Hima, A4: Phanta, S4: Phanta

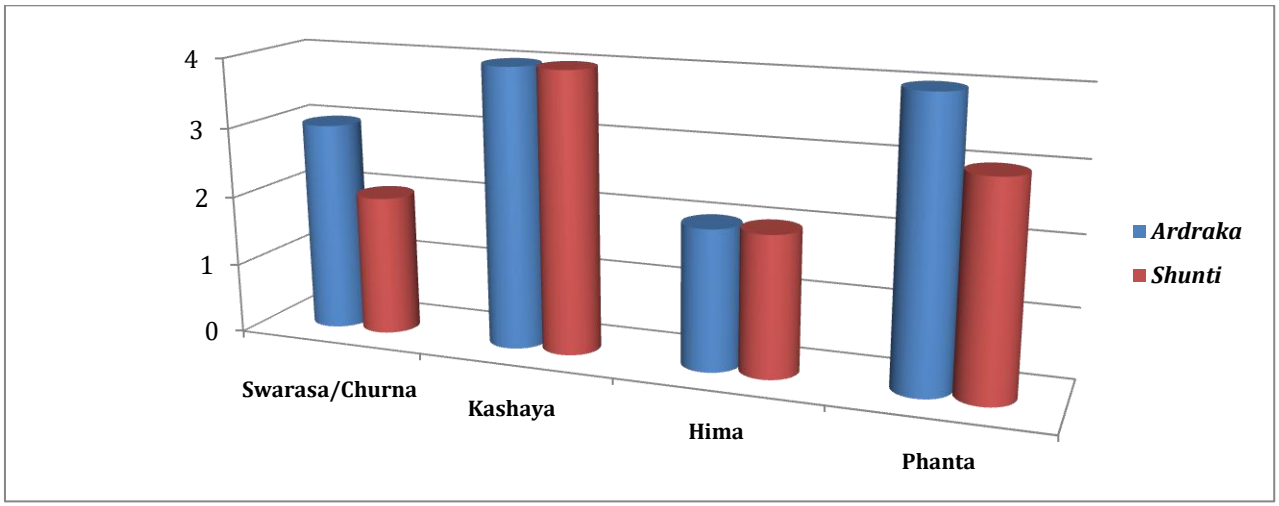


Fig 8: No. of peaks of *Ardraka* and *Shunti* At 254nm

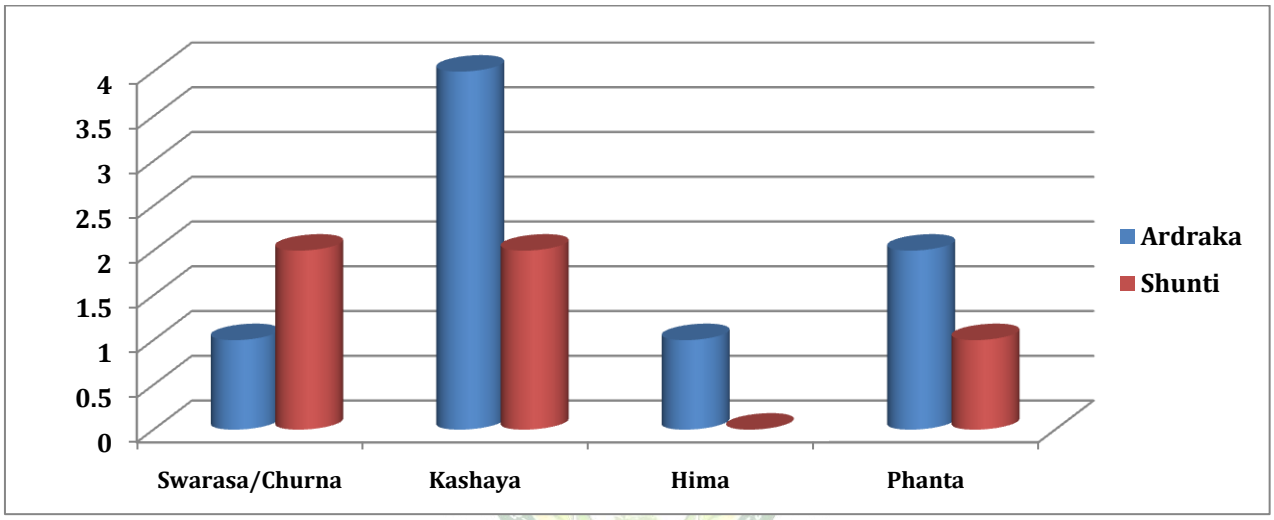


Fig 9: No. of peaks of *Ardraka* and *Shunti* at 366nm

Kashaya obtained from both ginger showed more number of peaks in 254nm. This might be due to the involvement of *Agni samskara* which might have extracted the aqueous soluble constituents. In 254nm and 366nm, there is a common peak at the maximum Rf value of around 0.6. In fresh ginger, another common RF value is found at 0.3 in 254nm. This indicates that few components can be extracted by all methods of preparation.

CONCLUSION

Based on HPTLC results, *Kashaya* from both *Ardraka* and *Shunti*; and *Phanta* from *Ardraka* have better extraction of constituents. As per phyto-chemical screening, *Ardraka swarasa* containing both alkaloids and flavonoids in abundance can be considered to be ideal for diabetes patients. However, further studies in HPTLC using bio-marker and anti-diabetic studies need to be conducted to confirm the same.

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