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Quantitative Analysis of Gallic and Ascorbic Acid in Fruit of *Draksha* (*Vitis vinifera* Linn) and *Kaashmari* (*Gmelina arborea* Roxb)

R G Raghi[1]* and Jollykutty Eapen[2]

[1-2]Department of Dravyagunavijnana, Government Ayurveda College, Thiruvananthapuram. Kerala, India

ABSTRACT

Objective: Over three quarters of world population relies on herbal products for health care, as they embody safety, concern to synthetics. Costly multivitamin preparations with several minerals are promoted as general tonics to keep good health and to protect from stress. This is irrational and wasteful. In order to maintain a proper health we should have the consort of natural products. *Draksha* and *kaashmari* fruits are taken to analysis the amount of ascorbic acid and gallic acid present and compared.

Method: Methanol extract of kashaya and sheeta kashaya of both the fruits where taken to analysis. HPTLC analysis was done after TLC to quantify the amount of gallic acid and ascorbic acid.

Result: In the analysis it was found that the amount of Gallic acid present in methanol extract of sheeta kasaya prepared with fruit of *Kaashmari* (GAW) was significantly greater than the samples GAKM, VVKM and VVW (P value<0.001). And in case of Ascorbic acid methanol extract of sheeta kasaya prepared with fruit of *Kaashmari* (GAF) was significantly greater than the GAKM, GAW, VVKM, VVF and VVW ($p<0.001$). Considering the products it was G.arborea which showed more amounts of Ascorbic acid as well as Gallic acid than V.vinifera in both the kashaya extracts.

Conclusion: On comparing the amounts of gallic and ascorbic acid fruit of G.arborea and showed more significance than fruit of V.vinifera.

KEYWORDS

Draksha, Kaashmari, Ascorbic acid, Gallic acid

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INTRODUCTION

Today over three quarters of world population rely on herbal products for health care, as they ensures safety when compared to synthetics. Nature is embedded with all sorts of problems and solution. When the body does not absorb or get enough amount of nutrient from food, nutritional deficiencies occurs. The fruits and vegetables which are cultivated in one's own land (where the person's birth happened) are the best to eliminate all sorts of pathologies and restore their health[1]. Commercial multivitamin preparations may contain 4-5 times the recommended dietary allowances. They are expensive and should be used only for deficiency states and during periods of increased vitamin requirement. They are not a substitute for a balanced diet. When these multivitamin tablets are used indiscriminately as dietary supplements to keep up the energy and strength, they act no better than placebos. The major portion of large doses of water-soluble vitamins is washed out in the urine within 24 hours. Often, costly multivitamin preparations with several minerals are promoted as general tonics to keep good health and to protect from stress; this is irrational and wasteful[2]. Inorder to maintain a proper health we should have the consort of natural products.

Draksha is well known drug since Vedic period. In Atharvaveda *draksha* in mentioned under the name of *krushana* and mentioned it for the management of *rajayakshama* (tuberculosis), *kilasa* (Leucoderma)[3]. Draksha is recommended as the best among the fruits in Ayurveda. Cultivated grapes are believed to have been introduced into the north of India by the Persian invaders in 1300 AD. Grape was also introduced in the south into salem and madurai districts of Tamil Nadu by the christian missionaries around 1832 AD[4]. Although grapes are cultivating widely, over use of pesticides and chemicals had degraded its quality[5]. Intake of such grapes for health is resulting in intricacies.

Kaashmari is the Sanskrit name of a medicinal plant mentioned in *ayurvedic* classics. It is one among the famous group of ten drugs, *Dasamoola*. It is a major ingredient of *ayurvedic* formulations like, *Chyavanaprasam, Dasamoolarishtam, Aravindasavam* etc. *Kaashmari* explained as the substitute for *Draksha* in classics[6]. As per Ayurvedic Pharmacopoeia of India (API), the source plant of *Kaashmari* is *Gmelina arborea* Roxb of Verbenaceae Family[7]. Gmelina roots are obviously famous, but its nutrient fruits are forgetten now a days. Its fruit is one among *madhura triphala* (three sweet fruits) along with the other two, *Draksha* (grapes) and *kharjura*.

(dates). It is *Balya* (Strengthening), *Brihmaneeya* (nourishing), *Rasayana* (rejuvenator), *Vrushya* (aphrodisiac) and indicated in *Vatapittaja Vikara, Raktapitta* (bleeding disorders), *Jwara* (fever) *Murccha* (fainting)[8] etc. Hence by quantifying and comparing the constituents in fruit of *Kaashmari* may make awareness among public thereby upgrade this fruit as consumptive one.

MATERIALS AND METHODS

Sample collection

Fruit of *draksha* (*Vitis vinifera* Linn) collected from its natural habitat organic farms, Kambam, Theni, and fruit of *kaashmari* (*Gmelina arborea* Roxb) collected from Government Ayurveda college Trivandrum and Tripunithura. Healthy fruits were washed with water and cleaned. Dried samples were then pounded and stored in air tight containers.

Sample preparation

Kashaya (decoction) and Sheeta kashaya (cold decoction) was prepared according to the decoction preparation procedure in Sarngadhara samhitha[9].

• *Kashaya* preparation -*Kashaya* (decoction) was prepared according to the procedures explained in Shargadhara samhitha. 48 g each of pounded dried fruit of *Draksha* (*Vitis vinifera* Linn) and *Kaashmari* (*Gmelina arborea* Roxb) were taken in different vessels. 192 ml water was poured in both and both are reduced to 48 ml and strained through clean white cloth (4 layered).

• *Sheetha kashaya* Preparation -*Sheetha kashaya* (decoction) was prepared according to the procedures explained in *Shargadhara samhitha*. Macerate 5 g of the coarsely powdered air dried drug and put in 30 ml of water taken in a beaker. Allow it to stand 12 hrs/ whole night, strained through clean white cloth (4 layered).

• From that prepared decoctions 10 ml was taken and dried under water bath. To the extract obtained 10 ml methanol was added and filtered out.

Prepared samples are shown in the figure no: 1.

Figure 1 Samples for Quantitative Analysis

Standard preparation

a) Preparation of standard solution of gallic acid:

A stock solution of gallic acid was prepared by dissolving 10 mg of gallic acid in 100 ml of methanol (0.10 mg/ml) was prepared.

b) Preparation of standard solution of ascorbic acid:

A stock solution of ascorbic acid was prepared by dissolving 10 mg of ascorbic acid in 100 ml of methanol (0.10 mg/ml) was prepared.

Standard samples were procured from Sigma Aldrich Bangalore.

Procedure

The solutions were applied to precoated, prewashed (methanol) and activated TLC plates as 6 mm wide bands, 15 mm from the bottom edge, 15 mm from side edge and 17 mm apart using a CAMAG Linomat 5 applicator. An application volume of gallic acid standards were spotted on tracks of plate$_1$ from 1 to 6 with volumes 3µl, 5 µl,7 µl,9 µl,11 µl,13 µl (i.e.,0.33 µg, 0.55µg, 0.77µg, 0.99µg, 1.21µg, 1.43µg) and ascorbic acid standards were spotted on tracks of plate$_2$ from 1 to 6 with volumes 2 µl, 4 µl, 6 µl, 8 µl, 10 µl, 12 µl (i.e.,0.2 µg, 0.4µg, 0.6µg, 0.7µg, 1µg, 1.24µg). 2 µl each of methanol extract of *draksha kashaya* (VVKM), *kaashmari kashaya* (GAKM), *draksha sheeta kashaya* (VVW), *kaashmari sheeta kashaya* (GAW) were spotted on tracks 7,8,9,10 of plate$_1$ and plate$_2$. After several trial and error, the mobile phase for gallic acid; toluene: ethyl acetate: formic acid: methanol (30:10:1:2) and for ascorbic acid; n-butanol: chloroform: acetic acid: ammonia: water (7:7:5:2:1) were used. Were the quantities of mobile phase was 10 mL.

The applied plates were run to solvent front of 70 mm by ascending development in twin through chamber at room temperature (25±2°C). After the development, TLC plates were dried in a current of air with a hair-dryer. The plate was observed under UV (254nm and 366nm) for detection followed by densitometric scanning. For obtaining calibration curve, densitometric scanning of the plate was performed in absorption mode at the wavelength of maximum absorbance. Quantitative data was obtained from the software by fixing the % deviation to get an appropriate regression line having a desirable standard deviation (<5) and regression coefficient (<1), while including maximum sample spots in the regression line. Percentage weight by weight (%w/w) of samples was calculated with respect to the % weight of drug in each kashaya.

RESULTS AND DISCUSSION

Gallic acid

a) HPTLC plate under UV lamp (photo documentation)

Upon viewing the developed HPTLC plate using the solvent system toluene: ethyl Acetate: formic acid: methanol (30:10:1:2) under UV at 254nm and 366nm, all the 4 samples showed bands (Fig no: 2).

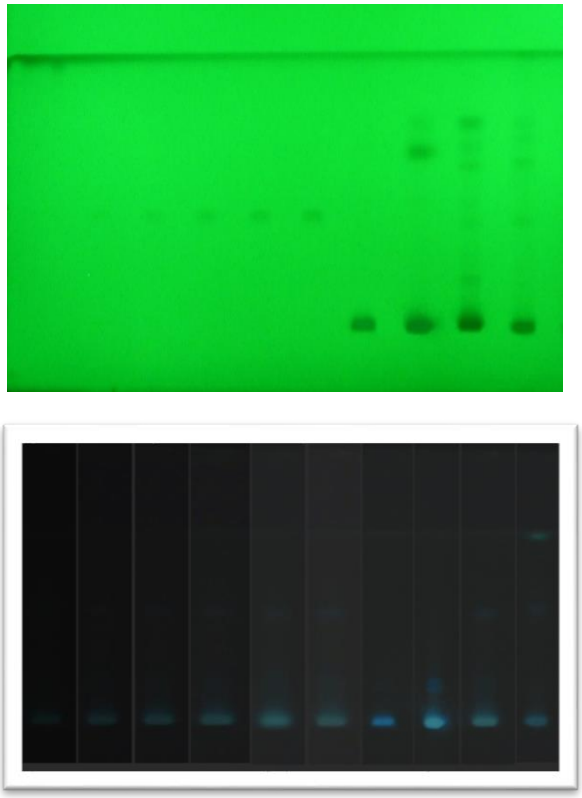
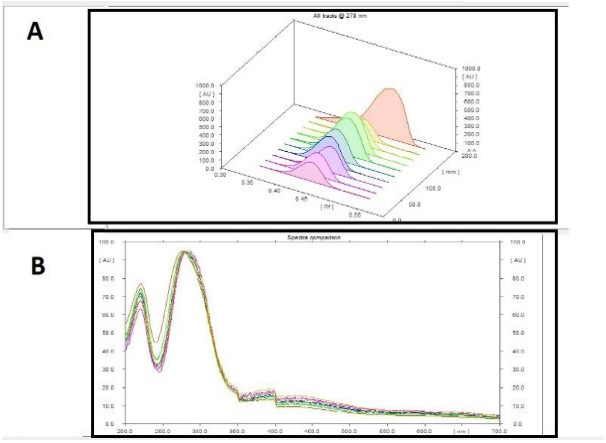


Figure 2 HPTLC Quantification of Gallic acid

b) Densitometric scanning

Densitometric scanning at 254 nm gave peaks of gallic acid at an Rf (Retention factor) value 0.44±0.0066. Among them, the peak heights were greatest for methanol extract of kaashmari sheeta kashaya (Fig no: 3).



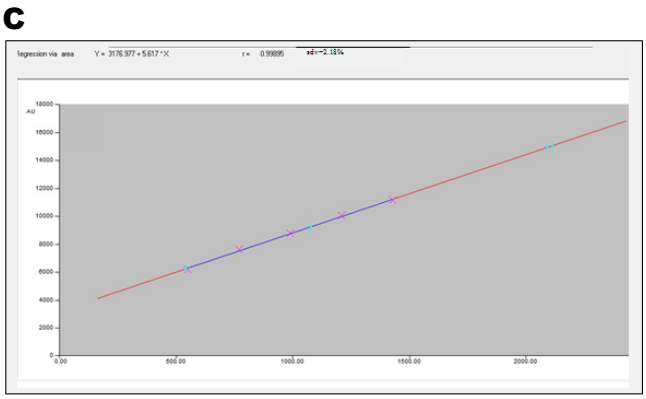


Figure 3 Gallic acid densitromeric scanning and spectral comparison

A.3D display of chromatogram for comparison of Gallic acid in standard solution and Gallic acid quantified from samples

B. Spectral comparison of standard Gallic acid and Gallic acid quantified from samples.

C. Calibration plot of Gallic acid using 6 different concentrations of GL verses peak area

c) *Spectral comparison of standard and samples (at 278 nm)*

Based on the initial spectral scanning, the spectral comparison was done at 278 nm, which was the wavelength of maximum absorbance. The spectra of standard and samples were superimposable, indicating the purity of gallic acid in samples.

d) Quantitation

A linear calibration curve was obtained both via peak area and peak height. The standard deviations and regression coefficients were desirable. The regression equation (via peak area), Y= 3176.977+5.617*x showed a standard deviation of 2.18% and a regression coefficient 0.9990. The graph for this equation is shown in fig no: 3.C. Three replicate analyses were done. The amount of gallic acid in samples was calculated

based on the quantitation of gallic acid in the WINCATS software, for the respective regression equations. It is expressed as % w/w of sample and given in table no: 1.

Table 1 Mean amount of gallic acid

SL no.	Sample	%w/w of Gallic acid (Mean ±SD)
1.	VVKM	$0.78*10^{-3}$±0.000
2.	GAKM	$1.23*10^{-3}$±0.0017
3.	VVW	$0.89*10^{-3}$±0.0005
4.	GAW	$1.56*10^{-3}$±0.0008

e) Validation studies

The method was validated for specificity, instrumental precision, linearity and accuracy, and the limit of detection and limit of quantification were calculated. The results are as depicted in table no: 2.

Table 2 Validation of study- gallic acid

SL no.	Experiment	Observation	Result
1.	Specificity	Rf of spot both in the standard and the sample were approximately same , with an overlapping spectra	Specific
2.	Instrumental precision	Rf of the spots= 0.44 ± 0.0066	Precise
3.	Linearity	Linear calibration curve was obtained both via height and area	Linear
4.	Accuracy	Inferred to be accurate as it was validated for precision, linearity and specificity	Accurate
5.	Limit of detection	-	1.28 ng
6.	Limit of quantitation	-	3.88 ng

f) Statistical analysis:

The differences between the mean quantities of gallic acid among the samples were tested statistically using one way ANOVA with post hoc test (Table no. 3, 4) and graphical representation as in figure no. 4.

Table 3 Anova test for gallic acid

SL no.	Sample name	Gallic acid		ANOVA P
1.		Mean	Sd	
2.	VVKM	0.78	0.000	<0.001
3.	GAKM	1.23	0.00017	
4.	VVW	0.89	0.0000	
	GAW	1.56	0.0000	

Table 4 Anova for gallic acid with post hoc comparison among groups

SL no.	Multiple comparison	Difference		P value	P-value Interpretation
1.		Mean	SE		
2.	VVKM *Vs* GAKM	0.45	0.0075	<0.001	***
3.	VVKM *Vs* VVW	0.10	0.0026	<0.001	***
4.	GAW *Vs* VVKM	-0.78	0.0023	<0.001	***
5	GAKM *Vs* VVW	-0.34	0.0042	<0.001	***
6	GAW *Vs* GAKM	-0.34	0.0045	<0.001	***
	GAW *Vs* VVW	-0.68	0.0002	<0.001	***

***: significant at 0.1% level (p<0.001).

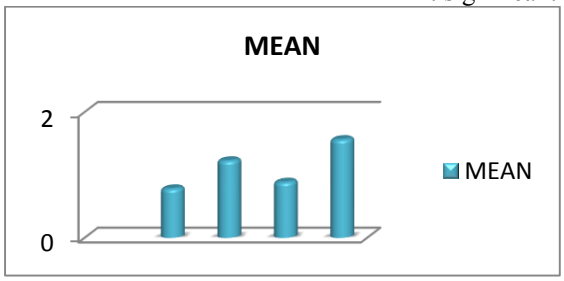


Figure 4 Mean amount of Gallic acid in samples

Ascorbic acid

a) *HPTLC plate under UV lamp (photo documentation)*

Upon viewing the developed HPTLC plate using the solvent system n-butanol + chloroform + acetic acid + ammonia +

water in the ratio (7:7:5:2:1) under UV at 254nm and 366nm, all the 4 samples showed bands at 254nm as shown in the figure.no.5.

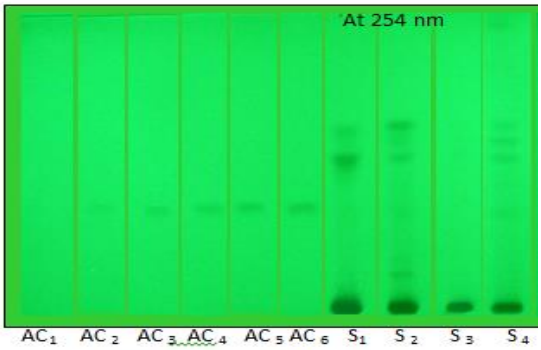
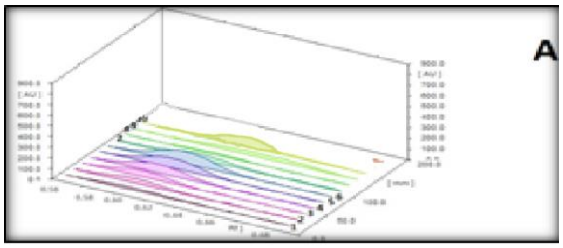


Figure 5 Quantification of Ascorbic acid HPTLC
AC_{1-6} Standard Ascorbic acid solution in different concentrations
S_1-VVK, S_2- GAK, S_3- VVW, S_4- GAW, S_5- VVF, S_6- GAF

b) *Densitometric scanning*

Densitometric scanning at 254 nm gave peaks of ascorbic acid at an Rf value 0.60 ± 0.013. Among them, the peak heights were greatest for methanol extract of kaashmari sheeta kashaya 2.12 mg/100g (Fig.no 6).



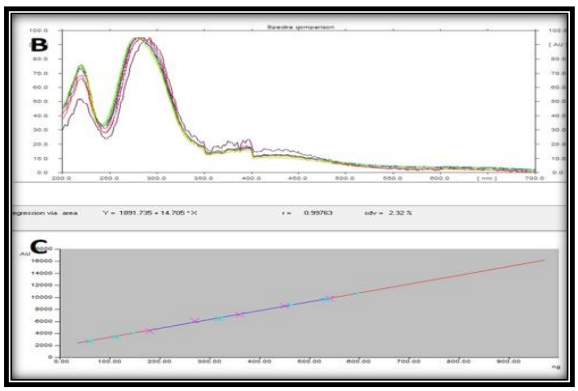


Figure 6 Ascorbic acid densitometric scanning and spectral comparison
A. Chromatogram comparison of Ascorbic acid in standard solution & samples
B. Spectral comparison of standard and Ascorbic acid quantified from samples.
C. Calibration plot using 6 different concentrations of Ascorbic acid verses peak area

c) *Spectral comparison of standard and samples (at 279 nm)*

Based on the initial spectral scanning, the spectral comparison was done at 279 nm, which was the wavelength of maximum absorbance. The spectra of standard and samples were superimposable, indicating the purity of ascorbic acid in samples.

d) *Quantitation*

A linear calibration curve was obtained both via peak area and peak height. The standard deviations and regression coefficients were desirable. The regression equation (via peak area), Y= 1891.735+14.705*X showed a standard deviation of 2.02% and a regression coefficient 0.99763. The graph for this equation is shown in figure no:6 C. Three replicate analyses were done. The amount of ascorbic acid in samples was calculated based on the quantitation of ascorbic acid in the WINCATS software, for the respective regression equations. It is expressed as % w/w of samples and given in the table no.5.

Table 5 Mean amount of ascorbic acid

SL no.	Sample	%w/w of Ascorbic acid (Mean ±SD)
1.	GAK	1.18± 0.03
2.	VVK	0.81± 0.02
3.	VVW	1.43± 0.01
4.	GAW	2.12± 0.11

e) Validation studies

The method was validated for specificity, instrumental precision, linearity and accuracy, and the limit of detection and limit of quantification were calculated. The results are as depicted in table no.6.

Table 6 Validation of study- ascorbic acid

SL no.	Experiment	Observation	Result
1.	Specificity	Rf of spot both in the standard and the sample were approximately same , with an overlapping spectra	Specific
2.	Instrumental precision	Rf of the spots= 0.60 ± 0.013	Precise
3.	Linearity	Linear calibration curve was obtained both via height and area	Linear
4.	Accuracy	Inferred to be accurate as it was validated for precision, linearity and specificity	Accurate
5.	Limit of detection	-	0.453
6.	Limit of quantitation	-	1.373

f) Statistical analysis:

The differences between the mean quantities of ascorbic acid among the samples were tested statistically using one way ANOVA with post hoc test (Table no. 7 & 8) graphical representation as in figure no: 7

Table 7 Anova test for ascorbic acid

SL.NO	Sample name	Ascorbic acid		ANOVA P
		Mean	Sd	
1	VVK	0.81	0.02	<0.001
2	GAK	1.18	0.03	
3	VVW	1.43	0.01	
4	GAW	2.12	0.11	

Table 8 Anova for ascorbic acid with post hoc comparison among groups

Multiple comparison	Difference		P value	P-value Interpretation
	Mean	SE		
GA KM *Vs* VVKM	-0.37	0.012504	<0.001	***
GAKM *Vs* VVW	0.25	0.017762	<0.001	***
GAW *Vs* VVW	-0.69	0.072616	<0.001	***
GAW *Vs* VVKM	-1.308	0.067358	<0.001	***
GAKM *Vs* GAW	0.936667	-0.05485	<0.001	***
VVKM *Vs* VVW	0.618	0.005258	<0.001	***

***: significant at 0.1% level (p<0.001).

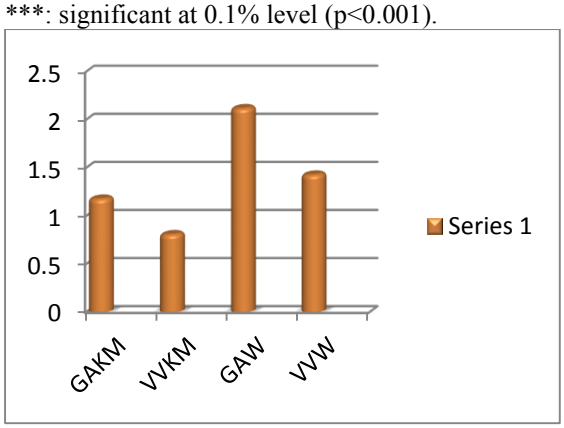


Figure 7 Mean amount of Ascorbic acid in samples

DISCUSSION

In HPTLC analysis, which was done after TLC to quantify the amount of gallic acid, it was found that the amount of Gallic acid present in methanol extract of *sheeta kasaya* prepared with fruit of *Kaashmari* (GAW) was significantly greater than the samples GAKM, VVKM and VVW (P

value<0.001) (as shown in the table no:4). And in case of Ascorbic acid methanol extract of *sheeta kasaya* prepared with fruit of *Kaashmari* (GAW) was significantly greater than the GAKM, VVKM, and VVW (p<0.001) (as shown in the table no: 8). Considering the products it was G.arborea which showed more amounts of Ascorbic acid as well as Gallic acid than V.vinifera in both the samples (GAKM & GAW, VVKM & VVW). Soaking of the drug had initiated better dissolvement of active principles to water than heating. This may be the reason for discussing *sheetha kashaya* for *draksha*[10] in specific ailments like murcha (fainting) etc .The solvent system "n-butanol: chloroform: acetic acid: ammonia: water (7:7:5:2:1)" for ascorbic acid which was not reported yet had shown good separation considering the other reported solvents. And this solvent system can further use in ascorbic acid quantification rather than the reported one.

CONCLUSION

The study entitled "Quantitative analysis of gallic and ascorbic acid in fruit of *draksha* (*Vitis vinifera* Linn) and *kaashmari* (*Gmelina arborea* Roxb)" was conducted to evaluate and compare the presence of ascorbic acid and gallic acid in fruit of *kaashmari* (*Gmelina arborea* Roxb) as it was explained as a substitute for *draksha* (*Vitis vinifera* Linn)

On comparing the amounts of gallic and ascorbic acid fruit of G.arborea and showed more significance than fruit of V.vinifera. And this data may increase the acceptance of *kaashmari phala* to public domain for daily consumption.

ABBREVATION

API- Ayurveda pharmacopeia of India

GA- *Gmelina arborea* Roxb

GAKM- *Gmelina arborea* Roxb kashaya

GAW- *Gmelina arborea* Roxb seeta kashaya

VV- *Vitis vinifera* Linn

VVKM- *Vitis vinifera* Linn kashaya

VVW- *Vitis vinifera* Linn seeta kashaya

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