

## “COMPARATIVE ANALYTICAL STUDY OF *MUSTADI KWATHA* AND *MUSTADI GHANA*, TWO DOSAGE FORMS OF A CLASSICAL AYURVEDIC FORMULATION”

Dr. Sharada Ramesh<sup>\*1</sup>, Supriya H. S.<sup>2</sup>, Sulochana Bhat<sup>3</sup>

<sup>1</sup>Senior Research Fellow (Ayurveda), CARI, Bengaluru.

<sup>2</sup>SRF (Chemistry) CARI, Bengaluru.

<sup>3</sup>Assistant Director in-Charge, CARI, Bengaluru.

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\*Corresponding Author: Dr. Sharada Ramesh

Senior Research Fellow (Ayurveda), CARI, Bengaluru.

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### ABSTRACT

**Background:** *Kwatha Kalpana* (Decoction), despite of its uniqueness and potency in ayurvedic pharmacopoeia, beholds certain drawbacks related to preparation, palatability, transportation etc. Practice of refrigerating the *Kwatha* is adopted considering the difficulty in fresh preparation. *Ghana* is one such dosage form wherein the intactness of the constituents can be expected with a longer shelf life. **Methods:** *Mustadi Kwatha* (MK) and *Mustadi Ghana* (MG) were prepared following the classical methods. MK was kept under two different temperatures, i.e. under room temperature (MKRT) and in the refrigerator (MKRF) for different durations, MKRT1 (24 hours) MKRT2 (48 hours), MKRF1 (24 hours), MKRF2 (48 hours), MKRF3 (96 hours) and MKRF4 (160 hours). Organoleptic characters were determined, phytochemical analysis including tests for alkaloids, flavonoids, phenolic compounds, glycosides, proteins and carbohydrates was carried out. HPTLC fingerprinting was performed on all the samples of MK and MG. **Results:** Yield percentage of MG was 2.86%. Organoleptic characters of MKRT changed by the end of 160 hours. MG was brownish black in color, had characteristic odor, astringent in taste, was hard and sticky. All the tested phytochemicals were present in both MK and MG. In HPTLC of MKRT and MKRF samples, R<sub>f</sub> ranged from 0.321 to 0.416. The common R<sub>f</sub> was noted to be 0.353, corresponded to gallic acid. Fluctuations were noticed in Area Under Curve (AUC) in MKRF samples suggesting changes in the constituents. **Conclusion:** *Mustadi Kwatha* is best used within 24 hours of preparation. Refrigeration can preserve *Mustadi Kwatha* longer, but potential chemical interactions may affect its constituents.

**KEY WORDS:** Mustadi Kwatha, Mustadi Ghana, refrigeration, storage, gallic acid.

## 1. INTRODUCTION

*Kwatha Kalpana* (decoction) is a key and extensively utilized dosage form in Ayurvedic medicine. It is prepared by simmering herbal drugs with water in a precise ratio, then reducing the mixture over moderate heat to achieve the desired concentration. *Kwatha* holds an advantage over other dosage forms due to its unique qualities, including adaptability, enhanced absorption and assimilation and the retention of numerous water-soluble components found in the raw materials. *Kwatha* is a highly effective and widely used dosage form, but it has certain drawbacks, such as the preparation is time-consuming and inconvenient, challenges in transportation and storage, and difficulties in prescribing an accurate dose. *Kwatha Dravyas* are predominantly *Tikta* (bitter) and *Kashya* (astringent) which makes it difficult for consumption thereby hindering treatment compliance.

Traditionally, *Kwatha* is intended for immediate consumption or to be used within a day. However, for convenience refrigerating *Kwatha* and consuming has come into practice as and when needed. The safety and effectiveness of this practice remains uncertain, and it is still unclear whether it can be recommended to consumers. Additionally, methods to extend the shelf life of *Kwatha* need to be explored. Numerous studies have been conducted to enhance its shelf life, improve its taste, and make its administration more convenient.

*Ghana* on the other hand, is a formulation where the active components can be stored for an extended period up to 3 years.<sup>[1]</sup> Acharya Sharangadhara was the first to describe shelf life of various formulations commonly used during those times.<sup>[2]</sup> Transforming *Mustadi Kwatha* into *Mustadi Ghana* and subsequently to *Mustadi Ghana Vati* could extend the formulation's shelf life. Additionally, this process will provide a defined shape and dosage form, facilitating easier administration, combats the challenges of palatability and hence leads to better compliance from the patients. This study explores the chemical makeup of *Mustadi Kwatha*-a classical *Kwatha* preparation in three different modes, freshly prepared, refrigerated at 4 degree and *Ghana*. Similarities and differences in their constituents are determined and focussed on their probable effects, with an objective of defining the possible pros and cons.

## 2. MATERIALS AND METHODS

### 2.1. Materials

#### Ingredients<sup>[3]</sup>

*Mustadi Kwatha* (KWC/MUS/2021-004) consists of *Musta* (*Cyperus rotundus* L.) rhizome, *Indrayava* (*Holarrhena antidysenterica* (Roth) Wall. ex A.DC.) seeds, *Devadaru* (*Cedrus deodara* (Roxb. ex D.Don) G.Don) heart wood, and fruits of *Haritaki* (*Terminalia chebula* Retz), *Vibhitaki* (*Terminalia bellirica* (Gaertn.) Roxb.) and *Amalaki* (*Phyllanthus emblica* L.) used in equal parts.

#### Chemicals and instruments

CAMAG HPTLC with an automatic sample applicator was used for the HPTLC analysis. TLC silica gel 60 F254 was used for the study. All the reagents used in the experiment were of analytical grade.

### 2.2. METHODS

#### 2.2.1: Preparation of dosage forms

##### Preparation and storage of *Kwatha*

*Kwatha* was prepared adopting the standard procedure mentioned in the classics.<sup>[4]</sup> Equal quantity *Kwatha* from the total yield, was filled in sterile airtight plastic containers and stored in 2 different temperatures i.e room temperature

(RT) and refrigerator i.e.  $<4^{\circ}\text{C}$  (RF). Each container was labelled separately. In RT, there were 2 batches of *Kwatha*. MKRT1 was opened after 24 hours of preparation and MKRT2 opened after 48 hours. In refrigerated sample there were four batches. MKRF1 (opened after 24 hours), MKRF2 (opened after 48 hours), MKRF3 (opened after 96 hours) and MKRF4 (opened after 160 hours).

### Preparation of *Ghana*

MK was prepared following standard procedure.<sup>[5]</sup> It underwent further boiling at a very low temperature of  $< 45^{\circ}\text{C}$  till complete removal of moisture. The resulting residue was carefully scraped off and subjected to 24 hours of drying in a dryer to assure complete moisture elimination. The resultant MG was meticulously preserved in a sterile container prior to analysis.

### 2.2.2. Analytical study

#### Organoleptic and phytochemical analysis

MKRT1, MKRT2, MKRF1, MKRF2, MKRF3, MKRF4 and MG were subjected to organoleptic, phytochemical tests including tests for alkaloids, flavonoids, phenolic compounds, glycosides, proteins and carbohydrates, as per the methods mentioned in Ayurvedic Pharmacopoeia of India.<sup>[6]</sup>

#### HPTLC analysis

HPTLC analysis was performed as per the standard procedure.<sup>[7]</sup> CAMAG HPTLC with an automatic sample applicator was used for the analysis. TLC silica gel 60 F254 was the stationary phase used. Toluene: ethyl acetate: formic acid in the ratio 5:5:1 served as the mobile phase. The samples were applied on a 10\*10 cm plate and chromatographic development was done in 10\*10 cm twin-trough chamber (TTC) where the mobile phase was pre-saturated for 20 minutes. After the chromatographic development, the plate was dried and placed in TLC visualizer 2 for photo-documentation under 254nm visible light. Meanwhile, the TLC plate was scanned with the help of TLC Scanner 4 to obtain the area under the curve and Rf values.

## 3. RESULTS

3.1. Yield percentage of MG: Yield was observed to be 12.86%

3.2. Organoleptic characters

Organoleptic characters of MK and MG are represented in Table 1.

3.3. Analytical study

3.3a. Phytochemical analysis (Qualitative)

The results of phytochemical analysis of MK stored for different durations under two different temperatures and MG are depicted in Table 2.

3.3b. HPTLC

HPTLC findings of MK stored at different temperatures and durations and MG are represented in Table 3 and 4 respectively.

Photo documentation and densitograms of MK and MG are depicted in Fig 1a, 1b, 1c, 1d.

Densitograms of MK stored under different temperatures for different durations are depicted in Fig 2a, 2b, 2c, 2d.

Area Under Curve (AUC) of Mustadi Kashaya (MK) and Mustadi Ghana (MG) in HPTLC analysis seen at different time intervals are represented in Graphh 1 and 2 respectively.

**Table 1: Organoleptic characters of MK and MG.**

		Color	Odor	Taste	Texture
<b>MK 0hr</b>		Dark brown	Characteristic	Astringent, bitter	Watery
<b>MK 24hr</b>	RT RF				
<b>MK 48hr</b>	RT		Slightly fermented odor	Astringent, bitter, slightly sour	Slightly slimy
	RF		Characteristic	Astringent, bitter	Watery
<b>MK 96hr</b>	RT	Dark brown solution with a superficial white layer	Strong fermented odor	Astringent, bitter, sour.	Slimy
	RF	Dark brown	Characteristic	Astringent, bitter	Watery
<b>MK 160hr</b>	RT	Dark brown solution with a thick white layer above	Putrid odor	-	Thick and slimy
	RF	Dark brown	Characteristic	Astringent, bitter	Watery
<b>MG</b>		Brownish black	Characteristic	Astringent	Sticky

\*MK= Mustadi Kwata, MG=Mustadi Ghana, RT=Room temperature, RF= Refrigerator

Table 1 represents the organoleptic characters of MK kept at different temperatures for different durations and MG.

**Table 2: Phytochemical profile of MK and MG.**

Sl No.	Parameters	Test	MK 0hr	MK 24hr	MK 48hr	MK 96hr	MK 160hr	MG 0hr
1	Alkaloids	Dragendroff's	✓	✓	✓	Traces	Traces	✓
2	Flavonoids	Alkaline reagent test	✓	✓	✓			✓
3	Phenolic compounds	Ferric chloride test	✓	✓	✓			✓
4	Glycosides	Keller Killani test	✓	✓	✓			✓
4	Proteins	Ninhydrin test	✓	✓	✓			✓
5	Carbohydrates	Molisch's test	✓	✓	✓		✓	

\*MK= Mustadi Kwata, MG=Mustadi Ghana.

Table 2 represents the phytochemical profile of MK kept at different temperatures for different durations and MG.

**Table 3: HPTLC findings of MK.**

Name of the sample	No. of spots		Maximum Rf value		Respective AUC	
	RT	RF	RT	RF	RT	RF
0 hour	6	-	0.358	-	0.02139	-
24 hour	5	8	0.416	0.411	0.02396	0.01708
48 hour	6	7	0.353	0.353	0.02701	0.02207
96 hour	7	7	0.321	0.303	0.03052	0.01853
160 hour	-	7	-	0.379	-	0.02393

\*RT= Room temperature, RF- Refrigerator, Rf- Retention factor, AUC= Area under curve

Table 3 represents the HPTLC findings of MK stored at different temperatures for different durations.

**Table 4: HPTLC findings of MG.**

Name of the sample	No. of spots	Maximum Rf value	AUC
Ghana	6	0.402	0.01181

\* Rf=Retention factor, AUC= Area under curve

Table 4 represents the HPTLC findings of MG.

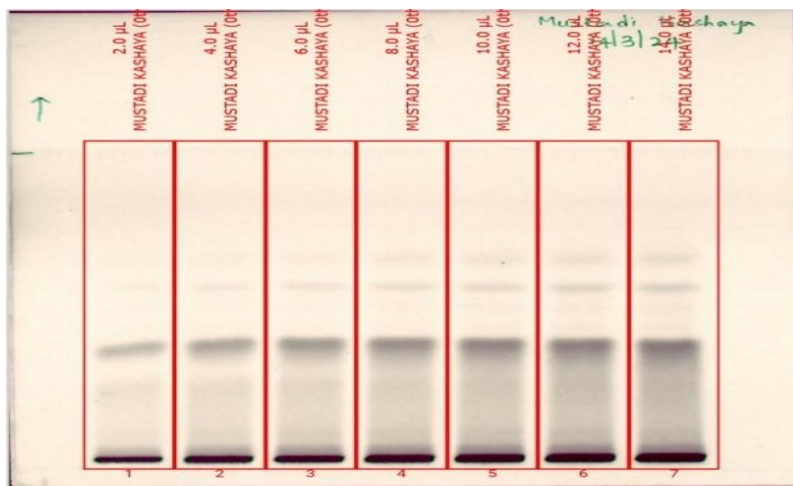


Fig 1a: Photo documentation of MK at concentrations 2, 4, 6, 8, 10, 12, 14μL visualized under 254nm.

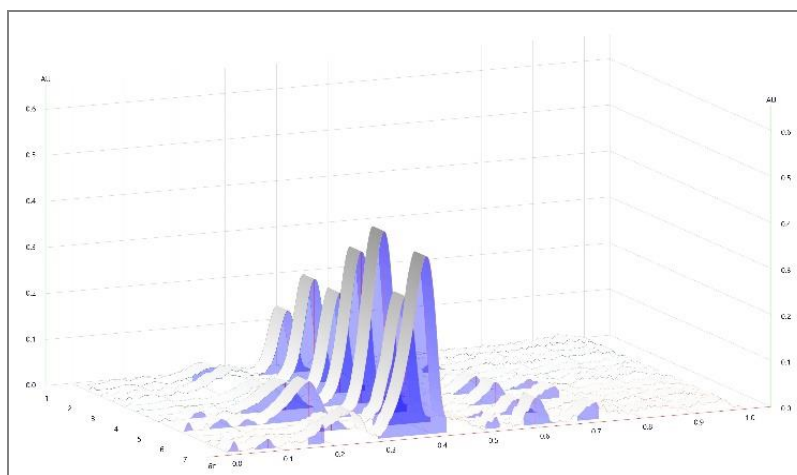


Fig 1b: Densitogram of MK at concentrations 2, 4, 6, 8, 10, 12, 14μL visualized under 254nm.

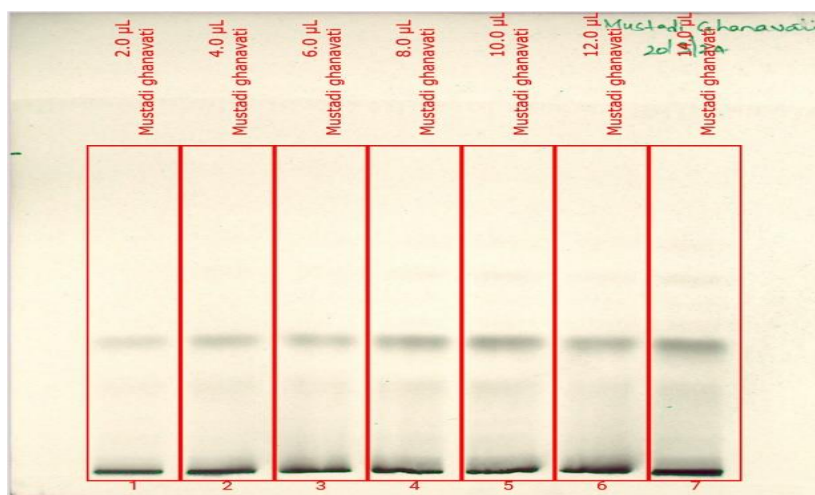
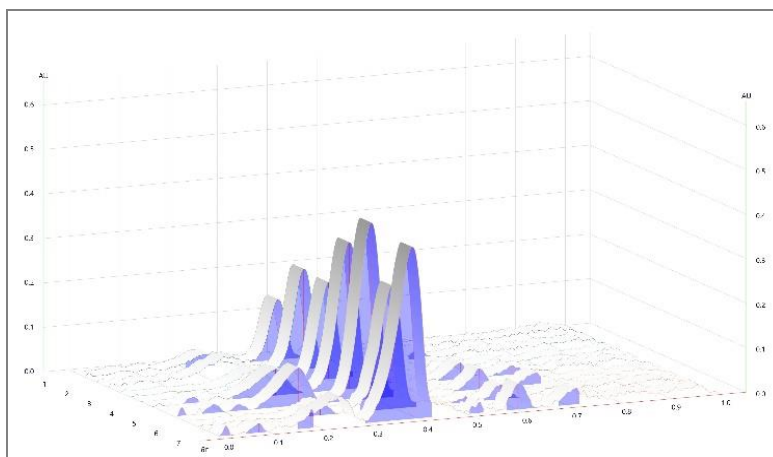
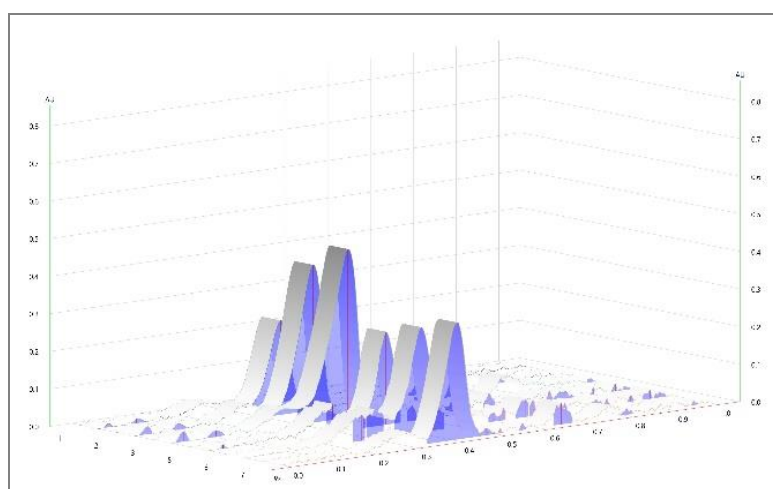


Fig 1c: Photo documentation of MG at concentrations 2, 4, 6, 8, 10, 12, 14μL visualized under 254nm.

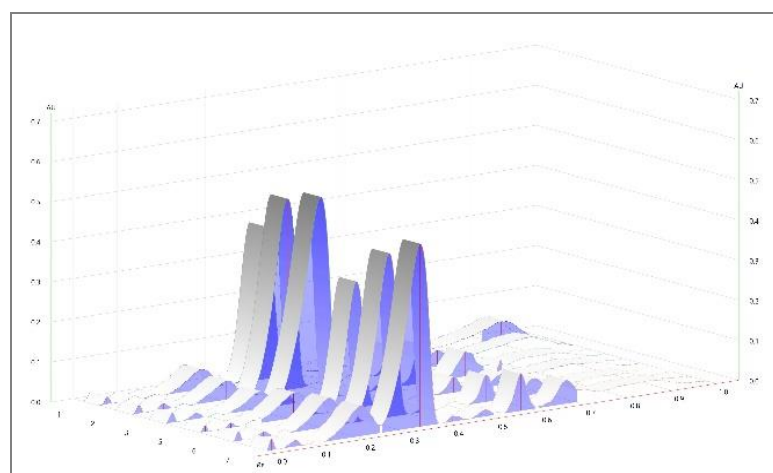


**Fig 1d: Densitogram of MG at concentrations 2, 4, 6, 8, 10, 12, 14 $\mu$ L visualized under 254nm.**



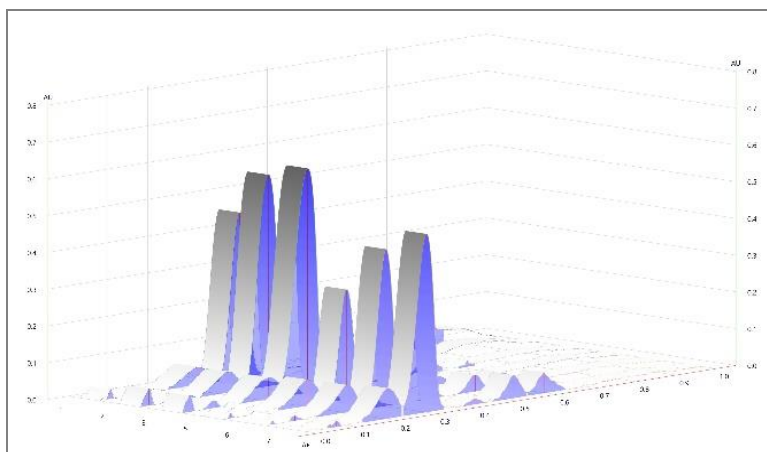
**Fig 2a: Densitogram of Mustadi Kashaya (MK) kept for a duration of 24 hours under room temperature and at 4° C at concentrations 2, 4, 6, 8, 10, 12, 14 $\mu$ L visualized under 254nm.**

Track 1,2,3 represents room temperature and Track 5,6,7 represents 4° C.



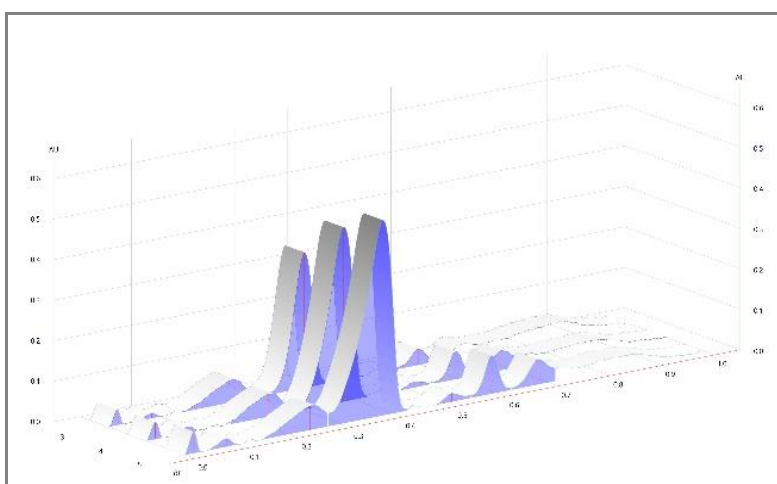
**Fig 2b: Densitogram of Mustadi Kashaya (MK) kept for a duration of 48 hours under room temperature and at 4° C at concentrations 2, 4, 6, 8, 10, 12, 14 $\mu$ L visualized under 254nm.**

Track 1,2,3 represents room temperature and Track 5,6,7 represents 4° C.



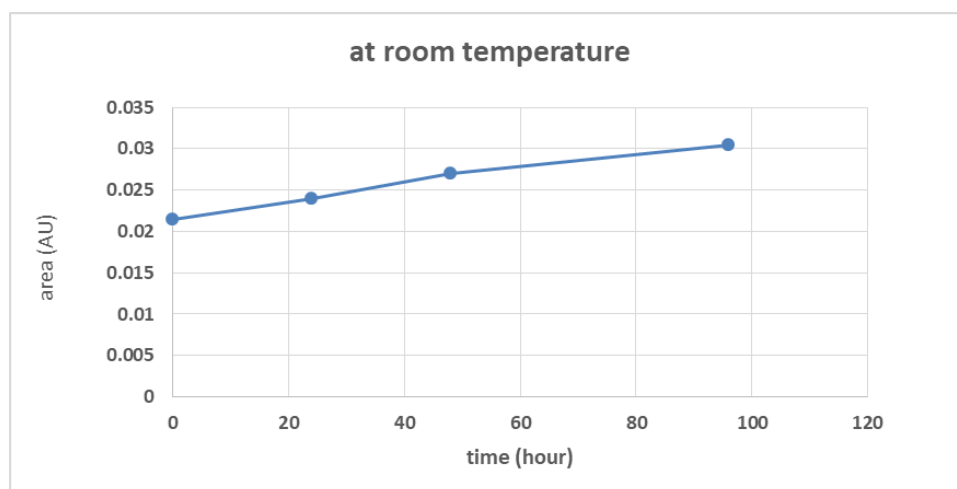
**Fig 2c: Densitogram of Mustadi Kashaya (MK) kept for a duration of 96 hours under room temperature and at 4°C at concentrations 2, 4, 6, 8, 10, 12, 14μL visualized under 254nm.**

Track 1,2,3 represents room temperature and Track 5,6,7 represents 4° C.

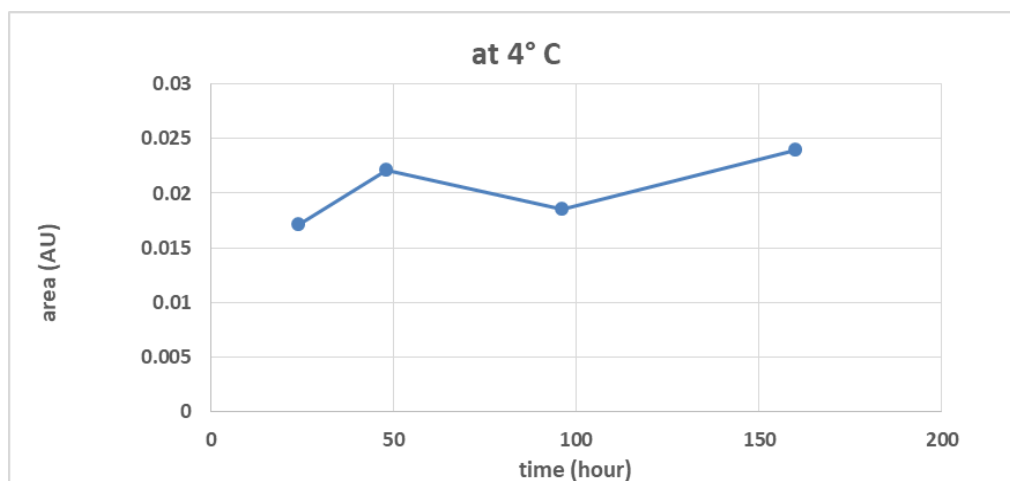


**Fig 2d: Densitogram of Mustadi Kashaya (MK) kept for a duration of 160 hours under room temperature and at 4° C at concentrations 2, 4, 6, 8, 10, 12, 14μL visualized under 254nm.**

Track 1,2,3 represents room temperature and Track 5,6,7 represents 4° C.



**Graph 1: Area Under Curve (AUC) of Mustadi Kashaya (MK) in HPTLC analysis seen at different time intervals.**



**Graph 2: Area Under Curve (AUC) of Mustadi Ghana (MG) in HPTLC analysis seen at different time intervals.**

## DISCUSSION

This work provides with an in-depth analysis of *Mustadi Kwatha* (MK) and *Mustadi Ghana* (MG), focusing on their organoleptic, phytochemical, and chromatographic properties under varying conditions. The yield of MG was 12.86%. The organoleptic properties of MK changed over time. Although color and texture remained largely stable, a sour taste developed after 24 hours and intensified by 48 hours. After 96 hours, complete putrefaction and fungal growth occurred, leading to the exclusion of further analysis. No significant qualitative changes in phytochemical properties of MK and MG were observed, but quantitative analysis might provide more insights.

In HPTLC analysis, peaks observed in MK samples (stored at room temperature and in refrigerator) at different time points showed variations, with room temperature samples displaying more variability. At room temperature the number of peaks at 0, 24, 48, and 96 hours were 6, 5, 6, and 7 respectively. In refrigerator, the number of peaks at 24, 48, 96, and 160 hours were 8, 7, 7, and 7 respectively. A common  $R_f$  value of 0.353 was noted in both room temperature and cold storage samples. The  $R_f$  obtained corresponds to the  $R_f$  of Gallic acid,<sup>[8,9]</sup> possibly indicating the presence of gallic acid, which is stable in both conditions. More peaks in cold-stored samples suggest better preservation of the chemical constituents. The consistent  $R_f$  values suggest the presence of gallic acid, which is a key phenolic compound in MK. Since gallic acid is heat-resistant,<sup>[10,11]</sup> MK can be stored for up to 24 hours at room temperature, but refrigeration introduces the potential for chemical interactions. Chemical interactions and changes in the composition of drug are evident in refrigeration. Storage at low temperatures can have positive as well as negative effect on phenolic compounds.<sup>[12,13]</sup> The fluctuating AUC (Area Under Curve) in refrigerated samples suggests dynamic chemical behaviour at low temperatures.

## Comparison Between MK and MG

MK stored at 4°C had more peaks than MG, suggesting that some constituents are lost during the conversion from *Kwatha* to *Ghana*. The  $R_f$  value of 0.402 in MG, matching gallic acid,<sup>[14]</sup> indicates that gallic acid is retained in the *Ghana* form, although the AUC decreased, implying a reduction in the active constituent's concentration.

**Limitations and scope for further research:** The dosage form consumes more preparation time, energy (fuel), and resources. The bulk production of *Mustadi Ghana* remains questionable from both an economic and environmental perspective. Given the decreased yield and reduced active components in *Mustadi Ghana*, fortifying the *Ghana* with an

equally potent *Kwatha* could potentially improve its efficacy. Punched tablets made from the aqueous extracts of MK can also be considered for testing.

## CONCLUSION

*Mustadi Kwata* is best used within 24 hours of preparation considering organoleptic and chemical stability, at room temperature. Refrigeration can preserve *Mustadi Kwatha* longer, but potential chemical interactions may affect its constituents. Gallic acid, a heat-resistant phenolic compound, plays a key role in the stability of *Mustadi Kwatha* and *Mustadi Ghana*, though its concentration decreases when the formulation changes from *Kwatha* to *Ghana*.

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