



## ORA - Analytical Study

### Quantification of total phenols and pharmacognostical evaluation of *Cuminum Cyminum* in different kashaya kalpanas using HPTLC fingerprinting

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#### ABSTRACT:

**Background:** *Cuminum cyminum L.*, known as *Jeeraka* is used as daily spice and flavouring agent in many cuisines. *Ayurveda* enlists "*Jeeraka*" as *deepana* and *pachana* drug. These seeds have stimulating, analgesic, and carminative properties. The present study provides well-designed and highly appropriate pre-clinical trial of three different aqueous extracts namely *kwatha* (decoction), *hima* (maceration), *phanta* (hot infusion) which differ in their procedures which produce variation in bioactive constituents of cumin. This study aims to quantify the total phenols in different preparations of *Cuminum cyminum* and further characterise the different formulation (*Kwatha*, *hima* and *phanta*) of *Cuminum Cyminum* by HPTLC analysis. **Methods:** This was an analytical study conducted in AYUSH accredited, Central Research Laboratory. The three drug sample of *kwatha*, *hima* and *phanta kalpana* of *jeeraka* required for the study were prepared as per the *Ayurveda Pharmacopoeia* and tested for its taxonomic authentication, phytochemical analysis, total phenolic content and pharmacognosy evaluation using HPTLC finger printing. **Results:** In the present study the Drug Sample A-*kwatha kalpana* had lesser phenolic content of  $1621.87 \pm 3.79 \mu\text{g}$  GAE/ml of sample when compared to the Drug Sample C- *Hima* having  $1892.17 \pm 1.05 \mu\text{g}$  GAE/ml and Drug Sample B-*Phanta* having  $2350.73 \pm 3.79 \mu\text{g}$  GAE/ml. The one-way ANOVA tests showed statistically significant difference between the samples for total phenolic content. Further the HPTLC for the 3 samples, in terms of maximum AUC (area under curve) and number of R<sub>f</sub> (retention factors) values, the sample prepared by the *Phanta* (hot infusion method), and *Kwatha* (decoction) appears to be the best. **Conclusion:** This study could identify the best form of aqueous extract of cumin are *phanta* and *kwatha* with better phytochemical properties and potential benefits.

**KEYWORDS:** Decoction, kwath, Jeeraka, Phenols, R<sub>f</sub> values, Cumin, HPTLC, TLC

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## 1. INTRODUCTION

Cumin is commonly known as *Cuminum cyminum* L., belongs to the Apiaceae (Umbelliferae) family. *Cuminum cyminum*L., known as 'Jeeraka' is used as daily spice and flavouring agent in many cuisines. The World Health Organization [WHO] estimates 80 percent of people worldwide use medicinal herbs for some form of primary healthcare. Numerous pharmacological properties, such as anticancer, antimicrobial, cardiovascular, antioxidant, anti-inflammatory, immunological, and analgesic effects, were demonstrated by plants. [1] Many cultures have utilized spices and herbs for thousands of years, and scientific studies have shown that they have antibacterial qualities. In many regions of the world, spices are also used in medicine, cosmetics, fragrances, and liquorices.

Cumin is a commonly used spice preservative and therapeutic agent. Apiaceae family includes the adaptable spice *Cuminum cyminum* L. (Jeeraka) as shown in figure no.1. This plant is native to Egypt, the Mediterranean, and South Asian nations, although having a pantropical spread. It has several traditional, medicinal, and culinary qualities hence used as a regular spice on daily basis in Indian food in different forms like cumin powder, cumin water, decoction. [2] This drug is used as medicine across systems of medicine to treat cases of gastrointestinal problems, urinary problems, eye disorder and inflammatory conditions. Cumin seeds have stimulating, analgesic, carminative, antimicrobial, insecticidal, anti-inflammatory, analgesic, antioxidant, anticancer, antidiabetic, antiplatelet aggregation, hypotensive, bronchodilator, immunological, contraceptive, anti-

amyloidogenic, anti-osteoporotic, protective and central nervous effects. In *Ayurveda* it is said to be *deepana* and *pachana*. [3]

Cumin seed's potential as a cancer chemopreventive agent may be related to its capacity to alter the metabolism of carcinogens. [4] The nonenzymatic glycation of proteins produces advanced glycation endproducts (AGEs), which are associated with aging and type 2 diabetes, among other health issues. Cumin was studied for its anti-glycative functions. [5] There are various forms of usage of cumin seeds namely *churna* (powder), *swarasa* (juice), *hima* (cold infusion), *kwatha* (decoctions), *phanta* (hot infusions), *grita* (processed ghee), *asava and arista* (fermentation forms) mentioned in classics of *Ayurveda*. [6], [7] Numerous bioactive, including terpenoids, flavonoids, and alkaloids, are found in *Cuminum cyminum* L. The primary bioactive that gives it the majority of its pharmacological and therapeutic relevance is cuminaldehyde. [8] The present study provides well-designed and highly appropriate pre-clinical trial of three different aqueous extracts which differ in their procedures which produce variation in bioactive constituents of cumin.

**Objectives:** This study aims to quantify the total phenols in different preparations of *Cuminum cyminum* L viz., *Kwatha*, *hima* and *phanta* and further characterise the different formulation (*Kwatha*, *hima* and *phanta*) of *Cuminum Cyminum* L. by HPTLC analysis.

## 2. MATERIALS AND METHODS:

This is an analytical experimental study which was piloted in AYUSH certified CRF laboratory (Central research facility) at KLE Academy of Higher Education

and Research Deemed-to-be University's Shri B.M.Kankanwadi Ayurveda Mahavidyalaya Belagavi where the authentication of drug was obtained with voucher no. BMK/CRF/19/2024-2025. The drug measurement and preparation was done as shown in Figure no. 2, Figure no. 3, evaluation of tests for phenols, HPTLC (High-Performance Thin-Layer Chromatography) analysis were conducted.

**Procuring Drug and authentication:**

In December 2023, the seeds of *Cuminum cyminum L.* were acquired from the authorized vendor. Central Research Facility, an AYUSH-certified laboratory in Belagavi, Karnataka, India, completed the taxonomic authentication. Both macroscopic and microscopic characteristics were studied in seeds as shown in Figure no.1. The plant material that was purchased was certified with authentication report, further, this was grounded into a coarse powder and utilized in accordance with established procedures to determine

the ash values, extractive values, and preliminary phytochemical analysis.

**Table no.1 Taxonomical Classification [9]**

Kingdom	Plantae
Subkingdom	Streptophyta-Seed plants
Superdivision	Spermatophyta - Seed plants
Division	Magnoliophyta - Flowering plants
Class	Magnoliopsida - Dicotyledons
Family	Apiaceae (Umbelliferae)
Genus	Cuminum
Species	Cuminum cyminum



**Figure no.1 Cuminum Cyminum Linn.**

**Table no.2: Ayurveda Parameters of Jeeraka as per classical references [10], [11], [12], [13], [14], [15]**

No	Properties& Actions	Criteria	
1.	Pharmacological Properties	<i>Rasa (taste)</i>	<i>Katu, Tikta</i>
		<i>Guna (attributes)</i>	<i>Ruksha, Laghu</i>
		<i>Veerya (potency)</i>	<i>Ushna</i>
		<i>Vipaka</i>	<i>Ushna Paka</i>
2	Pharmacological Actions	<i>On Humors (Doshaghnata)</i>	<i>Pittala , Vata-Kaphahara , Vatahara ,Kaphahara</i>
		<i>The mechanism (Karma)</i>	<i>Ruchya, Sangrahi, Chakshushya, Garbhashaya Vishodhana, Deepana , Medhya, Hrudya, Pachana , Jaranam, Vrushya, Balya</i>
		<i>Direct on diseases (Rogaghnata)</i>	<i>Chhardi ,Gulma ,Adhmana, Atisarahara , Grahani, Krumihara, Shopha, Jwaraghna, Ajeerna, Deepana, Pachana, Jarana, Chhardi, Adhmana, Atisarahara, Grahani, Ajeerna</i>
		<i>Action on Strotas</i>	<i>Rasa, rakta, Garbhashaya Vishodhana</i>

### Drug Samples Preparation Procedures:

As per Ayurveda Pharmacopeia of India (API) the three samples of the drugs were prepared. Drug A- *kwatha kalpana*(1:16):- the drug were weighed 10 grams (1 part cumin seeds) (Figure No. 2) and water (16 parts) which was boiled reduced to one-fourth of its original volume. 10gms of jeera was boiled in 160 ml of water and reduced to 40ml as shown in Figure No.3. Drug B- *Phanta Kalpana* where 1part drug (10gms seeds of *Jeeraka*) added to 6 times (60ml) of water at boiling stage and retained till it cools down and later filtered as shown in Figure no.4. The *jeeraka* seeds 1 part (10gms) are soaked in 6 times of water over night at room temperature and used in next morning time. The *jeeraka* water was strained to get *jeeraka Hima*. [16] These three drug samples were tested for phenols contents, HPTLC analysis.



Figure No. 2: Measurement of Jeeraka for the preparation of *Kalpana* samples



Figure no. 3: Preparation of Kwatha and *Phanta Kalpana*



Figure no 4: Filtration of *Phanta* and *Hima kalpan*  
Extraction of Phenolic Compounds:

**Total Phenolic Content (TPC) Assay:** Folin-Ciocalteu method (method using FC reagent) is a technique which measures the absorbance of phenolic chemicals found in natural products such as plant extracts using a microplate reader. FC assay was conducted in alkaline media; as phenolic compounds are reduced with the FC reagent. Concurrent with the process is the creation of a blue complex, whose peak absorbance was measured at 765 nm. The TPC (total phenolic contents) of the ethanoic and aqueous extracts were calculated using the FC reagent. The calibration curve was created by mixing 4.0 ml of sodium carbonate solution (75 g/l) with 5.0 ml of tenfold-diluted Folin-Ciocalteu reagent and 1 ml aliquots of 50, 100, 150, 200, 250, 300, 350, 400, and 450 g/ml Gallic acid solutions. After 30 minutes, the absorbance was measured at 765 nm. One millilitre of the ethanoic and aqueous extracts (1 gm/100 ml) was mixed separately with the same reagents to create the calibration curve. After an hour, the absorbance was calculated to determine the total phenolic content. [17]

High-Performance Thin-Layer Chromatography (HPTLC)

Drug forms are subjected to qualitative and quantitative analyses for their purity and efficacy using High-Performance Thin-Layer Chromatography (HPTLC) at KLE's Basic Science Research Centre, Belagavi. The drug sample needed a very concentrated solution, and the solvent on the plate is volatile and non-polar. The absorbent employed is silica gel pre-coats with tiny particle sizes. In the stationary phase, surplus solvent is removed from the plates by drying them in an oven at 120° C for 15 to 20 minutes after they have been cleaned with methanol. With a solvent or mobile phase in the proper container, the plate is arranged vertically. Chromatograms are produced on both sides of the capillary action-fed mobile phase (the sample spotted has a diameter of approximately 1 mm). Devices for recording data and computers are connected to the HPTLC instrument. Spot development is understood as maxima at specific UV region wavelengths. [17] The device measures the peaks' height and area, recording the results as a percentage

### 3. RESULTS:

**Macroscopic features:** The seeds of the *Jeera* (*cuminum l.*) plant were brown with light-coloured with ridges and a distinct aroma and flavour. It measured roughly 4-6 mm in length

**Physico-chemical parameters:** The organoleptic and physico-chemical characteristics of the plant components, which are reported in table 3, are another crucial diagnostic feature of the plants. Every parameter fell within the accepted bounds. The physical and chemical properties of *Cumin cyminum*

powdered seeds, including their ash value, acid-insoluble and water-soluble ash, alcohol, and water-soluble extractive, loss during drying, and foreign matter were analysed as shown in Table 3.

**Table 3: Physio-chemical parameters of seeds of *Cuminum cyminum***

Physio-chemical parameters	Values in %
Foreign Matter	0.224%
Loss on drying	2.569%
Ash value	6.429%
Ash soluble in Water	1.532%
Ash insoluble in Acid	0.575%
Water soluble extractive	20.464%
Alcohol soluble extractive	16.676%

The fluorescence analysis in different solvents was conducted in both normal and Ultra Violet (254 and 366 nm) light conditions. It was discovered that the pH values of 1% and 10% aqueous solutions were 5.90 and 5.45, respectively. Preliminary phytochemical analysis revealed the presence of proteins, amino acids, glycosides, tannins, and flavonoids in the petroleum ether, chloroform, ethanol, and aqueous extracts as shown in Table no. 4. Using toluene: ethyl acetate (7.5: 2.5) for the ethanoic extract and toluene: ethyl acetate (8: 2) for the aqueous extract as the mobile phase, respectively, TLC and HPTLC of the aqueous and ethanoic extracts were performed independently, and the R<sub>f</sub> (Retention factors) values were noted and illustrated (Figure no.5,6,7). Anisaldehyde-sulphuric acid reagent was used as a visualizing agent to produce the resolved spots' appearance.

**Table 4: Analytical study of Jeeraka in Kwatha, Phanta and Hima form**

	Drug Sample A- <i>Kwatha kalpana</i>	Drug Sample B- <i>Phanta Kalpana</i>	Drug Sample C- <i>Hima Kalpana</i>
Specific gravity	0.999	1.019	1.024
pH	5.88	5.53	5.63
Total solids	26.067%	17.125%	19.084%
Colour	Dark brown	Brown	Brown
Odour	Aromatic	Mild odour non Aromatic	Aromatic
Taste	Bitter	Bitter unfavourable	Slight bitter
Phenolic content	1621.87 ±3.79 µg GAE*/ml of sample	2350.73 ±3.79µg GAE/ml of sample	1892.17 ±1.05µg GAE/ml of sample

\*GAE -gallic acid equivalent

**Table 5: Rf values of various aqueous extract of cumin seeds**

Samples	^Rf values	Rf value (Max. AUC)*
<i>Phanta</i> (Hot infusion)	0.153, 0.256, 0.637, 0.934, 0.995	0.934, 0.637, 0.153
<i>Kwatha</i> (decoction)	0.152, 0.261, 0.677, 0.932, 0.992	0.152, 0.677, 0.932
<i>Hima</i> (cold infusion)	0.085, 0.250, 0.924, 1.000	0.085, 0.924, 1.000

\*Maximum Area under the curve, ^Rf – Retention factor

#### HPTLC analysis

The HPTLC analysis of *Cuminum cyminum* in three different *Kashaya Kalpanas*-*Phanta* (Hot infusion), *Kwatha* (Boiled decoction), and *Hima* (Cold infusion)—demonstrates distinct phytochemical profiles based on their **Rf values** and **area under the curve (AUC)**, indicating differences in extraction efficiency and constituent concentrations based on the preparation method as shown in Table 5.

**Phanta (Hot Infusion):** The HPTLC fingerprinting of the *Phanta* (hot infusion) extract of *Cuminum cyminum* revealed five Rf values: 0.153, 0.256, 0.637, 0.934, and 0.995. Among these, the most prominent peaks were observed at Rf 0.934 with 40.11% area under the curve (AUC), followed by Rf 0.637 (29.97%) and Rf 0.153 (16.75%). These results suggest that *Phanta* is capable of extracting both polar and moderately non-polar phytoconstituents effectively. The heat involved in this method likely enhances solubility, resulting in a diverse range of extracted compounds, particularly favouring those with higher Rf values (less polar in nature).

**Kwatha (Boiled Decoction):** In the *Kwatha* (boiled decoction) preparation, five Rf values were detected: 0.152, 0.261, 0.677, 0.932, and 0.992. The dominant peaks were at Rf 0.932 (33.05% AUC), 0.677 (28.44%), and 0.152 (18.39%). Although the Rf values and profiles are quite similar to those in *Phanta*, the intensities of the peaks show slight variations. This indicates that the decoction process effectively extracts a wide range of constituents similar to hot infusion, but the boiling process may lead to partial

degradation or transformation of some thermolabile compounds.

**Hima (Cold Infusion):** The HPTLC fingerprint of *Hima* (cold infusion) displayed four  $R_f$  values 0.085, 0.250, 0.924, and 1.000. The major constituents were found at  $R_f$  0.924 with the highest AUC of 44.30%, followed by  $R_f$  1.000 (30.89%) and  $R_f$  0.085 (15.64%). This indicates that cold infusion is efficient in selectively extracting certain phytoconstituents, especially non-polar compounds. However, the overall number of detectable compounds is lower, suggesting a narrower phytochemical profile. Despite the absence of heat, this method still demonstrates significant extraction efficiency for specific compounds, preserving delicate or heat-sensitive constituents.

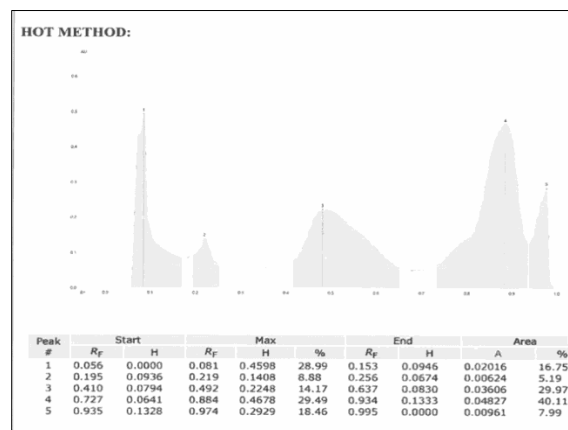


Figure no.5: HPTLC Finger printing of *Phanta* (Hot infusion) extract of seeds of *Cuminum cyminum L.* scanned at wavelength 366nm

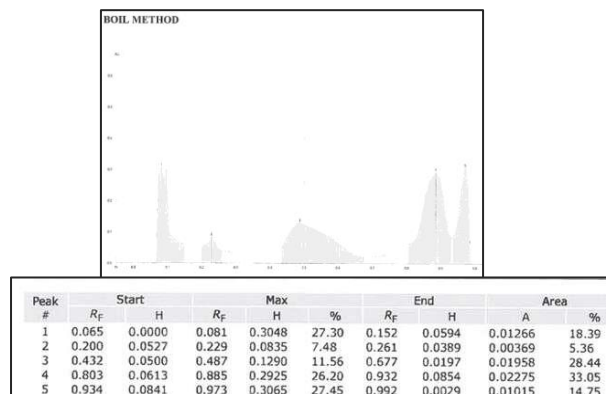


Figure no.6 HPTLC Finger printing of *Kwatha* (Decoction) extract of seeds of *Cuminum cyminum L.* scanned at wavelength 366nm

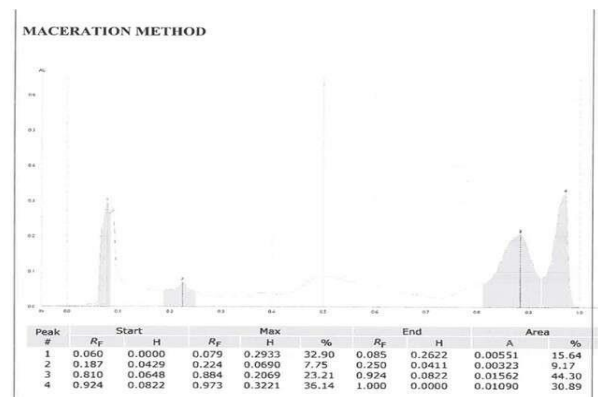


Figure no.7: HPTLC Finger printing of *Hima* (Cold infusion/ maceration) extract of seeds of *Cuminum cyminum L.* scanned at wavelength 366nm and 2 mm in width

**Statistical analysis:** In this study, the values obtained from total phenolic content (TPC) assay by Folin-Ciocalteu method was statistically analysed by using One-way ANOVA tests in GraphPad Prism version 8.0.2 manufactured by Graph Pad software, Inc. from California, USA. The phenolic contents of the sample are shown in figure no.8

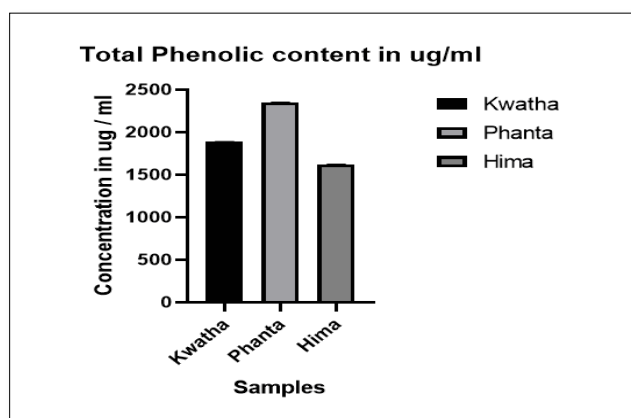


Figure no.8: The graph of total phenolic content (TPC) assay

**Table 6: One Way ANOVA results**

ANOVA table	SS *	DF <sup>†</sup>	MS <sup>‡</sup>	F *	P value <sup>  </sup>
Treatment (between columns)	814571	2	407285	F (2, 6) = 40945	P<0.0001
Residual (within columns)	59.68	6	9.947		
Total	814631	8			

\*SS-sum of squares, †DF-degree of freedom, ‡ MS- mean squares, \*F-statistics from distribution table, ||P-value- probability of observing if null hypothesis is true.

The one-way ANOVA test performed revealed statistical significant difference in mean phenolic content between groups of the sample at a p-value of 0.0001 (F2,6) = 40945, p = 0.0001) as shown in table 6.

#### 4. DISCUSSION:

Herbal medications must be processed into appropriate form for a particular action because they cannot be utilized in their raw state. The *Kashaya kalpana* is a process or modification that transforms the drug into various medicines to enhance the drug potency and palatability. The *Swarasa* (Juice of the drug), *Kalka*(paste), *Kwatha* (Boiled method), *hima* (cold infusion) and *phanta* (hot infusion) are the five types of basic *kashaya kalpana* mentioned in *Ayurveda* classics which are *guru* (heavy for digestion) in ascending order. In *swarasa*, the water-soluble extracts of the drug are extracted, the scientific reasoning being the particles of whole drug in *Swarasa* form are more grinded with the water present in the fresh drug or added water. Hence, the medicine with increased *kleda* (water) becomes more heavy for digestion. In *kalka* (paste) is a product that contains whole plants along with cellular waste and indigestible parts like starch, so it has a lower concentration than *swarasa*. It is made by extracting water-soluble and heat-stable ingredients from hard and woody medicinal plants, and the amount of water is determined by the hardness of the medicine used. The

*kwatha* which is well processed will have the smell, colour and taste as per the ingredients. In *Hima kalpana*, that drugs having *sheeta virya* (cold potency) and volatile principles are soaked in water and used. This is specially for drugs who lose their active ingredients by heating. In *Phanta Kalpana* the drug is soaked in hot water, hence easiest for digestion. [18]

This research focused on finding the best form of *kalpana* to obtain the active components of *Jeeraka*. Appropriate drug identification and authentication are essential for research and the healthcare system today. The raw drug and the final product were within allowable limits, guaranteeing the drugs' purity and proving that there was no microbial contamination. Using sense organs to observe drug's properties is known as organoleptic character analysis. This determines some of the material's unique properties and is the first step in determining the material's type and purity level. The phenolic content analysis provided valuable information about the antioxidant potential and health-promoting properties of cumin. Earlier studies by Rizvi observed higher phenolic content is generally associated with greater antioxidant activity and potential health benefits. The results of phenolic content analysis can be used to optimize extraction methods and evaluate the effects of processing or storage on phenolic content, and

compare the quality of different cumin samples or varieties. [19] Earlier studies by Meena et.al mentioned the presence of volatile oil like hydrocarbon cymol which acts as antioxidant, estrogenic in activities and further the cumin seeds are anticonvulsant, carminative, antibacterial in action. [20], [21] In this study the Drug Sample A- *kwatha kalpana* had lesser phenolic content of  $1621.87 \pm 3.79 \mu\text{g GAE/ml}$  of sample when compared to the Drug Sample C- *Hima* having  $1892.17 \pm 1.05 \mu\text{g GAE/ml}$  and Drug Sample B-*Phanta* having  $2350.73 \pm 3.79 \mu\text{g GAE/ml}$ . This effect may be due to boiling of drug in *kwatha kalpana* where the original drug quantity gets reduced to  $\frac{1}{4}$ <sup>th</sup> part which may lead to evaporation of volatile oils of cumin. In the present study the phenolic content analysis plays a crucial role in characterizing the bioactive components of cumin and understanding its potential health effects. It provides valuable insights for researchers, food scientists, and manufacturers in the development of cumin-based products with enhanced nutritional and functional properties.

The HPTLC fingerprinting confirms that different *Kashaya Kalpana* methods influence the qualitative and quantitative phytochemical composition, helping in selecting the appropriate preparation for targeted therapeutic effects. *Hima Kalpana* sample showed the highest single AUC peak (44.30%), suggesting that cold infusion may selectively extract certain potent constituents more efficiently. *Phanta* and *Kwatha* exhibited broader phytochemical profiles, with several major peaks, making them potentially more suitable when a wider spectrum of active constituents is desired. The similarity in Rf values between study

samples and prior findings by Meena et al. (e.g., Rf 0.67, 0.77, 0.73, 0.11) supports the standardization and authenticity of the *Cuminum cyminum* samples used. Hence the results showed *Phanta kalpana* (hot infusion) had more phenolic contents and exhibited broader phytochemical profiles which may be due to the effect of drug powder of cumin is soaked in water at boiling point and closed where the volatile oils did not evaporate and retained till cooled. The study shows the presence of various phenols, cumin aldehyde, terpenes, phenols, and flavonoid with different retention factor (Rf) values and areas under curve which confirms the presence of respective compounds. ] procedures.

**Relevance of the findings:** In terms of AUC (area under curve) among the three samples, the Rf values were best obtained in the *Phanta* (hot infusion) method. Hence, in clinical trials and treatment plans which require more antioxidant properties of the Cumin, the *phanta kalpana* of cumin can be used for better results. There is requirement of documentation and research of the best form of drug suitable in specific clinical conditions. These results underscore the importance of selecting appropriate extraction methods to maximize the therapeutic potential of cumin. This research study was an effort to analyse the best aqueous extraction of Cumin seeds.

**Limitations:** This study has not analysed the volatile oil contents of the samples. Further the other *kalpanas* like *swarasa* (juice), *churna*(powder), *arista kalpana* can be studied and analysed.

## 5. CONCLUSION:

This study highlights the significant impact of extraction methods on the phenolic content and

chemical composition of *Cuminum cyminum*. The *Phanta* preparation, involving hot infusion, retained the highest phenolic content, suggesting its superiority in preserving bioactive compounds. The HPTLC analysis further characterized the distinct chemical profiles of each preparation, emphasizing the presence of essential bioactive constituents. These results underscore the importance of selecting appropriate extraction methods to maximize the therapeutic potential of cumin. Based on the peak and area we concluded that boil and hot method are better than cold infusions. Future studies should focus on pre-clinical and clinical evaluations of these preparations to fully elucidate their health benefits and applications in traditional and modern medicine.

**Abbreviations:**

HPTLC- High-Performance Thin-Layer Chromatography

CRF: Central Research facility

BSRC- KLE's Basic Science Research Centre, Belagavi

API: Ayurveda Pharmacopeia of India

GAE -Gallic acid equivalent

TPC: Total Phenolic Content

WHO: World Health Organization

FC reagent: Folin-Ciocalteu reagent

Max. AUC: Maximum Area under the curve

Rf-Retention factor

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Approval of final manuscript: All authors.

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